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ORIGINAL ARTICLE

The Novel Synthesis Route for 3-Poly (vinylbenzyl)-5,5-Dimethylimidazolidine-2,4-dione Nanofibers and Study of Its Antibacterial Properties

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KEYWORDS

N-halamine nanofibers;
Electrospinning process;
Antimicrobial agent;
Antibacterial activity;
Decontamination

ABSTRACT: As part of our ongoing interest in exploring the synthesis of antibacterial agent, we report an efficient, mild, one pot and chemoselective procedure for preparation of 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione as a antimicrobial agent from poly(vinylbenzyl chloride) and 5,5-dimethylhydantoin in the presence of a base in DMF as a solven. The FT-IR technique and melting point confirmed the desire product. Then, 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers was produced by electrospinning process. To confirm the nanofiber materials, SEM image was applied. Then, these nanofibers were, chlorinated with sodium hypochlorite. Finally, the antibacterial activity of synthesized nanofibers was evaluated by against *Staphylococcus aureus* as gram-positive bacteria and *Escherichia coli* as gram-negative bacteria. The results from culture media and halo diameters of nanofibers showed that high biocidal effect of fibers against these bacteria. These nanofibes could be used in order to construct filters needed to clean and disinfect drinking water in emergency.

INTRODUCTION

The transmission of bacteria from the environment to a person is linked to the ability of the microbe to survive on the fabric surfaces. Neely and Co-workers reported the survival of 22 multiple Gram-positive bacteria on the hospital fabrics [1]. All isolated bacteria survived for 1 day, and other survived 90 days on the various fabrics. Another study examined the length of survival of various gram-negative bacteria on fabrics and plastics commonly used in hospitals [2]. Several gram-negative bacteria like *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonine* were tested in this study. At 102 bacteria per swatch, survival ranged from 1 h to 8 d. These bacteria survived from 2 h to more than 60 d, at 104 to 105 microorganisms per swatch. In order to protect the patients against these biological pathogens in the hospitals, much effort has been applied to the

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development of infection-resistant fibrous materials used in the hospital [3-9]. Biocidal functions, coated on fibrous materials, are one of the most widely used approaches to eliminate these bacteria. Among the biocidal agents, N-halamines structures have been a class of durable and effective antimicrobial agents with broad inhibitory activities [10-12]. On the other hand, nano fiber materials are widely used to eliminate microbial agents due to their high surface to volume ratio [13]. Recently, few reports have been reported on the medical uses of nanofibers.

In this study, first, we prepared and characterized 3-Poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione, and, second, electrospined and final nanofiber product was produced. In the final stage, the antibacterial characteristic of synthesized nanofiber was evaluated against Gram positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria by paper disc diffusion method.

MATERIALS AND METHODS

Materials and Characterization

All commercially available chemicals were obtained from Merck and Fluka companies, and used without further purification unless otherwise stated. The morphology of the gold-sputtered electrospun nanofibers were observed by scanning electron microscope (SEM) (Hitachi S-4160, Japan). The average fiber diameter (AFD) and distribution was determined from selected SEM image by measuring at least 50 random fibers using Image J software (National Institute of Health, USA). The infrared spectra of samples were recorded on a Perkin Elmer Spectrum 100 in the wave number range of 500-4000 cm^{-1}; a nominal resolution for all spectra was 4 cm^{-1}.

Microorganisms

The antibacterial property of synthesized 3-Poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers was carried out using two different bacterial strains including gram negative *E. coli* and gram positive *S. aureus*. All bacterial strains were provided from Biology Department, Imam Hossein University, Iran. Shaker incubator was purchased from Jal Company.

General procedure for the synthesis of 3-Poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione

A 100 mL round bottom flask was equipped with a rubber septum, magnetic stir bar and was charged with 12.8 gr of 5,5-dimethylhydantoin (0.1 mol) and potassium carbonate (0.29 mol, 40 gr) in 40 ml DMF at 40 °C for 5 min. After then, poly (vinylbenzyl chloride) (0.025 mol, 4.5 gr) were added to the mixture at room temperature for 24 h. After the reaction was complete, the water was added to the mixture to precipitate the white solid residue. Finally, the white solid product was filtered, collected and washed with 20 ml methanol [14].

Preparation of 3-Poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers

3-Poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione (5-10 wt.%) was dissolved in DMAC for electrospinning process via magnetic stirrer for 24 h at 50 °C. The electrospinning device used in this experiment was produced by Fanavaran Nano Meghyas Co. (Iran). To produce electrospun nanofibers, the polymer solutions were placed into a 3 mL plastic syringe with an 18 gauge (diameter=0.12 mm) needle tip as a nozzle for electrospinning. A syringe pump fed polymer solution to the needle tip. The metallic needle was connected to a positive high voltage and the collector (aluminum foil) was connected to the ground. The grounded collector was located at different distances (12-18 cm). All electrospinning experiments were carried out at room temperature and spinning conditions such as electric potentials, polymer percent, spinning distance, and solvents were optimized to obtain good nanofibers. Nanofiber was chlorinated by exposing the treated material to an aqueous solution of 0.6% NaOCl for 30 min. The nanofiber were then rinsed thoroughly with deionized water and dried overnight at room temperature.

Microbiological culture

The antibacterial activity of 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofiber was examined against *E. coli* and *S. aureus* bacteria by disk diffusion method according to Hwang and Ma procedure [15]. This method measures the effects of antimicrobial agents on bacteria growth in a culture. Muller-Hinton Agar (MHA) powder was applied as culture medium for bacteria growth. Nineteen grams of agar was dissolved into 500 ml of distilled water to prepare the culture medium, and then the solution was boiled to obtain transparent brown solvent. To achieve sterilized MHA medium, it was heated at 120 °C for 1 h in autoclave, then cooled to room temperature and poured into sterilized petri dishes (10-90 mm). The bacteria were wiped out uniformly across the culture plate after cooling over 24 h. Finally, filter-paper disks were placed on the surface of agar.

Evaluation of antibacterial activity

0.1 ml of nanofiber water suspension each samples were dropped moderately on disks' surface using a sampler to estimate antibacterial activity. If the samples were effective against the bacteria at a certain concentration, then no colonies would grow and the concentration in agar was greater than or equal to the effective concentration. This region is called zone of inhibition (ZOI). The size of ZOI measures the efficacy of antibacterial efficacy. A more effective sample produces a larger clear area around the disk. All tests were done under laminar flow hood. Finally, all petri dishes containing bacteria and antibacterial reagents were incubated at 37 °C for 24 h.

Physical and spectroscopic data

Spectra data of poly (vinylbenzyl chloride):

White solid powder; **mp** 145°C; **IR** (KBr) (ν_{max}, cm^{-1}) 1444 and 1264 (C=C), 706 (C-Cl).

Spectra data of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione:

white solid (86% yield); **mp** 180°C; **IR** (KBr) (ν_{max}, cm^{-1}) 3400 (N-H), 1701 (C=O), 1441 (C=C).

RESULTS AND DISCUSSION

Optimization of the reaction conditions

As part of our ongoing interest in exploring the synthesis of antibacterial agent [16 and 17], herein we report a novel, efficient, mild, one pot and chemoselective procedure for preparation of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione as a antimicrobial agent from poly (vinylbenzyl chloride) in the presence of K_2CO_3 as base in DMF as a solvent (Figure 1).

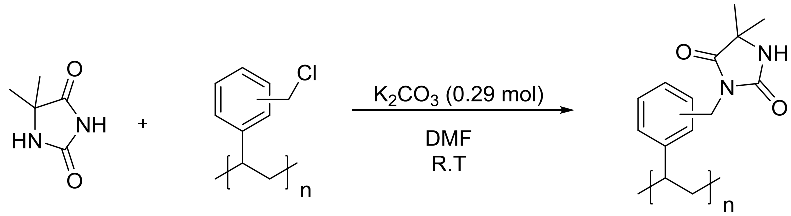

Figure 1. One pot synthesis of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione

To optimize the reaction conditions, base, solvents and temperatures of the reaction were investigated. Initially, we decided to explore our base in DMF and acetone for the synthesis of 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione (Table 1, entries 1 and 2). K_2CO_3 and DMF were the best base and solvent respectively. In the absence of base, no desire product was observed. Temperature of the reaction was investigated and gratifyingly, the best result was obtained at room temperature (entries 2-4) which is a very important point in organic synthesis especially in green chemistry in terms of environmental and economic aspects.

Table 1. Optimization of the reaction conditions.

Entry	Solvent	Base	Temperature (°C)	Yield (%)
1	DMF	K_2CO_3 (0.29)	R.T	86
2	Acetone	K_2CO_3 (0.29)	R.T	42
3	DMSO	sodium bicarbonate(0.29)	100	61
4	DMF	K_2CO_3 (0.29)	85	31
5	Dioxane	-	R.T	0
6	DMF	K_2CO_3 (0.1)	R.T	51

FT-IR spectroscopy

FT-IR technique was applied to confirm the synthesis of 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione. The FT-IR spectra of poly (vinylbenzyl chloride) are shown in Figure 2 (1). The band at 706 cm^{-1} was attributed to C-Cl asymmetric stretching mode. The C=C bond of benzene cycle in the starting material was appeared at 1444 and 1264 cm^{-1} related to streaching vibration. The formation of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione was confirmed according to Figure 2 (***2***) , thereby, the sharp peak at 706 cm^{-1} (C-Cl) in Figure 2 (1) was eliminated due to substitution of N-H with Cl. The broad peak at 3400 cm^{-1} (N-H stretching mode) is another confirmation for desire product.

SEM analysis

Electric potential of 22 kV and 13 cm distance between the syringe and the collector surface was applied to gain the best nanofibers. SEM images of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers are shown in Figure 3. The average diameter of nanofibers was about 300 nm.

Antibacterial properties nanofibers

3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers were chlorinated with sodium hypochlorite. The efficacy of nanofibers on the two types of bacteria includes *S. aureus* and *E. coli* are shown in Figure 4. The inhibition zone around nanofibers disks was measured in mm (Table 2) with ruler. These nanofibers have high antibacterial activity against both gram-positive (*S. aureus*) and negative (*E. coli*) bacteria. However, comparing two images showed that the effects of nanofibers are higher against gram-positive bacteria.

Direct effects of nanofibers on *E. coli* in culture represented complete eradication of bacteria in first 20 min. As shown in Table 2, nanofibers can kill both *E. coli* and *S. bacteria*. According to diameters of the formed halos around these bacteria, it could be realized that *E. coli* is less sensitive to the chlorine released from

N-halamine 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 designing of protective clothing that are resistant against biological agents used in unconventional warfare. These nanofibers could be used in order to construct filters needed to clean and disinfect drinking water in emergency.

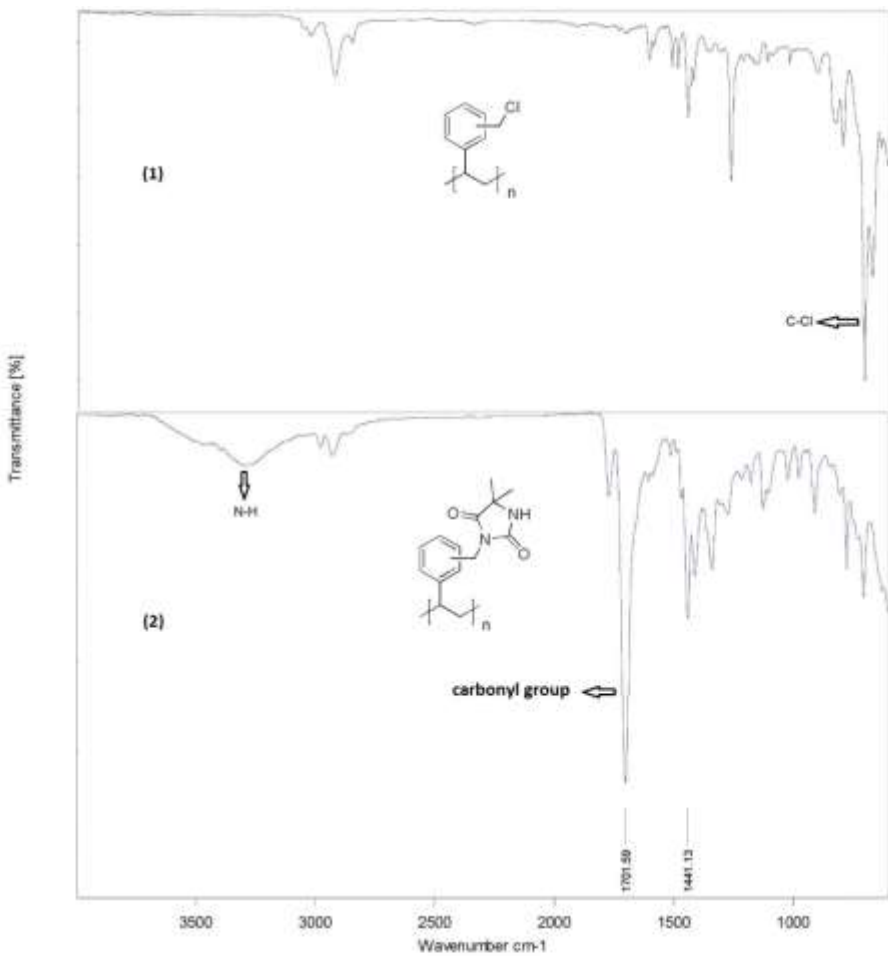

Figure 2. FT-IR spectra of poly (vinylbenzyl chloride) (1), 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione (2).

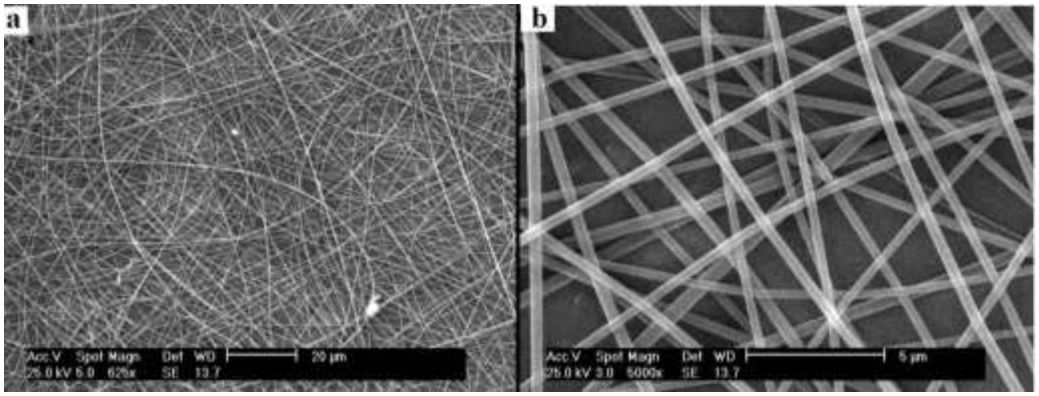

Figure 3. SEM image of 3-poly (vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione nanofibers: a) magnification with 675 times, b) magnification with 5000 times.

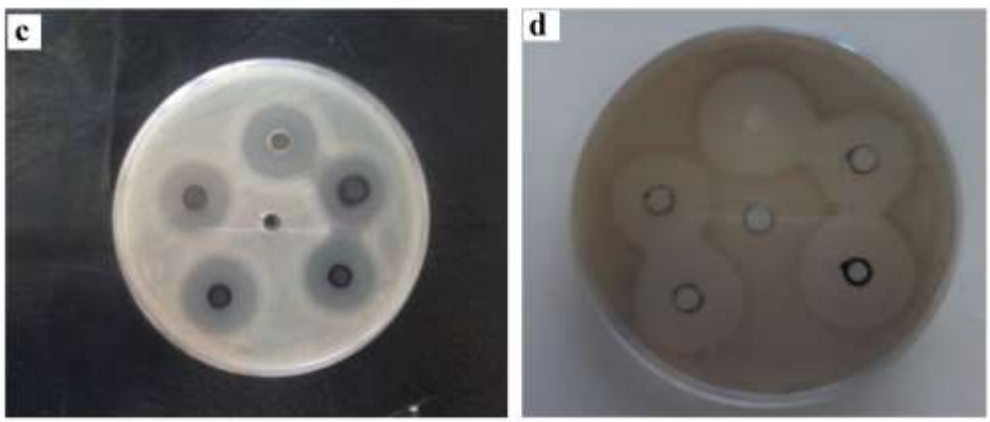

Figure 4. Schematic illustration of created inhibition zones for antibacterial activity of nanofibers against c) Escherichia coli, d) Staphylococcus aureus.

Table 2. Halo diameters of nanofibers in presence of Escherichia coli and Staphylococcusaureus in the culture media (mm)

Sample	**Formation diagonal circulars**		
	Nanofiber weight (mg)	**Escherichia coli**	**Staphylococcusaureus**
0	0.0010	0 mm	0 mm
1	0.0020	7 mm	10 mm
2	0.0040	14 mm	20 mm
3	0.0060	20 mm	26 mm
4	0.0100	22 mm	Non-measurable

CONCLUSIONS

We have developed a facile protocol for the synthesis of 3-poly(vinylbenzyl)-5,5-dimethylimidazolidine-2,4-dione from the reaction of 5,5-dimethylhydantoin with poly(vinylbenzyl chloride). After that, the synthesized product was electrospinned to achieve desire nanofibers. In the final stage, the antibacterial activity of corresponding nanofibers was examined by disk diffusion method. High ability of nanofibers in elimination of both gram-positive and negative bacteria. This nanofiber could be used in hospital equipment, to prevent the spread of common hospital infection. Moreover, these nanofibers could be used in order to construct filters needed to clean and disinfect drinking water in emergency.

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