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Cationic Copolymers Based On Styrene And N,N-Dimethylaminoethyl Methacrylate For Antibacterial Activity Applications

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Abstract

Cationic copolymers based on styrene and N,N-dimethylaminoethyl methacrylate (DMAEMA) were synthesized. Styrene was reacted with DMAEMA through conventional free radical polymerization. This reaction was performed using styrene to DMAEMA ratios of 1:0.25, 1:0.5, 1:1, and 1:2 moles to form copolymers SD0.25, SD0.5, SD1, and SD2, respectively. The resultant copolymers were characterized by Fourier transforms infrared (FTIR) spectroscopy, and proton nuclear magnetic resonance (1HNMR) spectroscopy. The surface morphology was studied using field emission scanning electron microscope (FESEM). The positive charge was introduced to the formed copolymers through alkylation with hexyl bromide forming the corresponding cationic copolymers; CSD0.25, CSD0.5, CSD1, and CSD2, respectively. The cationic copolymers were characterized by FTIR, 1HNMR, wide angle X-ray diffraction (WAXRD), thermogravimetric analysis (TGA) and FESEM. The antibacterial activity of the copoylmers and their cationic forms were tested against Escherichia coli serotype O145 (E. ColiO145) and Staphylococcus aureus (S. aureus) strains. CSD0.5 and CSD1 were found to have a promising antibacterial activity against both bacterial strains.

Keywords: Styrene; N,N-Dimethylaminoethyl methacrylate; Cationic; Antibacterial.

1. Introduction

The vast usage of polymeric materials in different fields of life pushes the scientists to work hardly to develop these materials to meet specific requirements of the application environment. These applications include textile industry [1], food packaging [2], and medical devices [3] that can simply be infected by fungi or bacteria, often producing severe medical troubles. One of the major advantages of the polymeric material is the ability to destroy bacteria. This ability is attained by either introducing physically antibiotic or bioactive small molecules to the polymer. Leaching of these small molecules to the working environment is useful for destroying bacterial colonization. However, the sustainability of the releasing process is weekend by time due to the consumption of the bioactive molecules [4-8]. An alternative method to make the polymer self-antimicrobial material is to chemically modify it by covalent bonding the active molecule or to convert the polymer to poly quaternary ammonium or phosphonium salts [9].

This method offers a permanent killing effect [10-14]. Quaternary ammonium salts are widely utilized as cationic antibacterial materials [15]. The most accepted mechanism of killing bacteria by polymeric quaternary ammonium salts is: (a) adherer and penetration of polycations onto wall of the bacterial cell, (b) reaction and destroying the cytoplasmic membrane, (c) outflow of cell constituents (proteins and nucleic acids) [16,17]. In addition, polymeric quaternary ammonium salts give more chemical stability, prolonged antimicrobial efficiency and less toxic residue, many examples of polymeric quaternary ammonium salts have been extensively reported [18-20]. Polystyrene (PS) is an interesting thermoplastic polymer with excellent mechanical and physical properties that render it one of the most applicable polymer in verities of applications in addition to its low production cost [21]. Modification/functionalization of PS is mostly done to attain it special property or to overcome any physical or mechanical shortage in addition to increase its resistivity to microbes.

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Modification of PS is achieved by chemical method [22], irrigation method, grafting or copolymerization with other monomers [23]. DMAEMA is characterized by possessing numerous active centers like double bond, ester group and amino functionality, so it is considered as highly beneficial multifunctional monomer. Copolymerization with active monomers leads to obtain water-soluble polymers, that can be used as flocculants in water treatment either potable or waste [24]. It has notable applications in biology and medicine due to its biocompatibility [25,26]. Besides, it improves bulk plastic materials properties either by grafting or copolymerization such as polyethylene and polypropylene [27,28]. Copolymerization of styrene with DMAEMA was reported a long ago using either conventional or controlled radical polymerization. Polystyrene-block-poly N,N-dimethylaminoethyl methacrylate was prepared by ATRP and their self-assembly behavior was considered [29]. Stable Free radical polymerization (SFRP) [30] and RAFT polymerization [31] were also studied. Herein, we are interested in preparation of copolymers of styrene and N,N-dimethylaminoethyl methacrylate monomers through conventional free radical polymerization followed by alkylation with hexyl bromide to form the corresponding cationic copolymers. The thermal stability and surface morphology of the produced cationic copolymers are studied. Antibacterial activity are also tested against both Staphylococcus aureus (S. aureus) and Escherichia coliO145 (E.Coli) strains.

2. Experimental

2.1. Materials

Styrene and DMAEMA monomers were purchased from Sigma. Both monomers were purified before use by passing over silica gel column. Azobisisobutyronitrile (AIBN) and hexyl bromide were bought from Aldrich. Tetrahydrofuran (THF), and chloroform ($CHCl_3$) were obtained from Merck.

2.2. Instruments

Spectrophotometer model Shimadzu 8400, Japan, was used for detecting the FTIR spectra of materials. The samples were measured in the spectral range between 400 and 4000 cm-1. 1HNMR spectra were conducted on a Jeol-Ex-500 NMR spectrometer and chemical shifts were stated as part per million. XRD measurements were performed on Philip's X-ray diffractometer PW1390 with Ni-filtered CuKα radiation (wavelength of 1.5404 Å) at generator voltage and tube current of 40 KV and 30 A, respectively. The diffraction angle 2θ was scanned at a rate of 2˚ min-1. TGA was conducted with Shimadzu TGA-50H. The measurements were performed at a heating rate of 10˚C min-1 from 30 to 600˚C under nitrogen atmosphere. Morphology of copolymers surface was considered by FESEM model QUANTA FEG 250 ESEM.

2.3. Synthesis of copolymers of styrene and N,N-dimethylaminoethyl methacrylate monomers

A mixture of styrene and DMAEMA monomers was reacted under argon atmosphere in THF in presence of AIBN initiator. The reaction continued for 6 hours at 70 °C. Styrene and DMAEMA were copolymerized using different ratios; 1:0.25, 1:0.5, 1:1, and 1:2 moles, respectively. The reaction mixtures were precipitated by methanol to give the copolymers SD0.25, SD0.5, SD1, and SD2, respectively according to the used percentage. The products were precipitated in methanol and allowed to dry at 40 oC for 48 h.

2.4. Synthesis of cationic styrene-co-N,N-dimethylaminoethyl methacrylate

SD0.25, SD0.5, SD1, and SD2 were dissolved in CHCl3 and reacted with hexyl bromide at 60 °C for 4 hours. The obtained products; CSD0.25, CSD0.5, CSD1, and CSD2, respectively; were washed several times with n-hexane for solidification.

2.5. Biological activity against Staphylococcus aureus (Gram positive bacteria) and Escherichia coliO145 (Gram negative bacteria)

2.5.1. Bacterial isolation and identification

Bacterial strains that used in this study were isolated from animal food origin (meat products) [32] as follow: 1gm of sample was injected to 10 ml of peptone water under complete aseptic conditions, strongly shacked, and then incubated at 37 oC for 8 to 12 hours. Ten microliters of the sample have been spreaded on the mannitol salt agar plate then incubated for 18 to 24 hours at 37 oC on the MacConkey agar plate. Colony morphology which was selected from mannitol salt agar plate was named as staphylococci, Gram staining, oxidase test, catalase test and oxidative fermentative test. The enzyme coagulase was analyzed using both the slide

and tube methods after the Staphylococcus genus was tested. Staphylococci isolates have been identified at the stage of the species using the commercial identification method API Staph (API Staph bioMerieux ® SA 69280 marcy- 1'Etoile/ France) [33,34] which were recognized as S. aureus (Gram positive bacteria) while colonies selected from MacConkey agar plate were known as E. coli depending on colony morphology, Gram staining, oxidase test and examined biochemically by methyl red, indole, Voges-Proskauer and Simmons citrate (IMVic) tests as conclusively E. coli (Gram negative bacteria). Consequently, the strains were recognized by the biochemical test system API 20E (Biomerieux) according to the manufacturer's commands, and serologically classified as E. coli serotype O145. Identified strains were sub-cultured and moved to Todd Hewitt broth (Becton Dickinson Diagnostic Systems, Sparks, MD), grownup at 37 oC for 18 hours, and stored in 20 % glycerol broth at -80 oC until use [35].

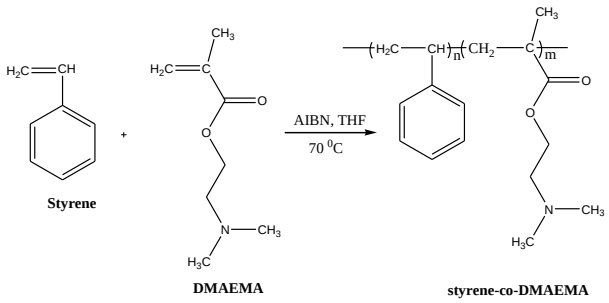
2.5.2. Screening of antibacterial activity using disk diffusion test

The antibacterial activity of poly(styrene-co-N,N-dimethylaminoethyl methacrylate) copolymers and their cationic forms was studied using a Mueller-Hinton agar culture medium as it was described in the disk diffusion test [36,37]. Bacteria that are freshly cultured were injected in saline solution, and the level of turbidity was attuned to standard McFarland 0.5. Suspensions of bacteria were inoculated and perfectly spread on microbiological Petri dishes containing Mueller-Hinton agar medium, then 5 mm in diameter wells were located on the inoculated test organisms and filled with samples at different concentrations (10 up to 50 µg/ml). Petri dishes were incubated at 37 °C for 24 hours. Inhibition zones were determined to evaluate the antimicrobial activity. Bacteria examined against several concentrations of each sample were prepared in triplicate every time.

3. Results and discussions

3.1. Synthesis of copolymers of styrene and N,N-dimethylaminoethyl methacrylate monomers

Styrene and DMAEMA were copolymerized as represented in Scheme 1. The reaction was conducted in THF by AIBN initiator at 70 °C under argon atmosphere using ratios 1:0.25, 1:0.5, 1:1, and 1:2 moles to form copolymers SD0.25, SD0.5, SD1, and SD2, respectively. The obtained copolymers were characterized by FTIR, 1HNMR, and FESEM. FTIR spectra illustrated in Fig. 1 show the presence of a vibrational band around 3025 cm-1 attributed to unsaturated =CH stretching. The two bands at 2933 and 2858 cm-1 are attributed to asymmetric and symmetric stretching vibrations of saturated –CH. The band observed at around 1728 cm-1 is corresponding to ester group stretching. The located band at around 1631 cm-1 is attributed to C=C aromatic ring vibrations.



Scheme 1 Synthesis of styrene-co-DMAEMA.

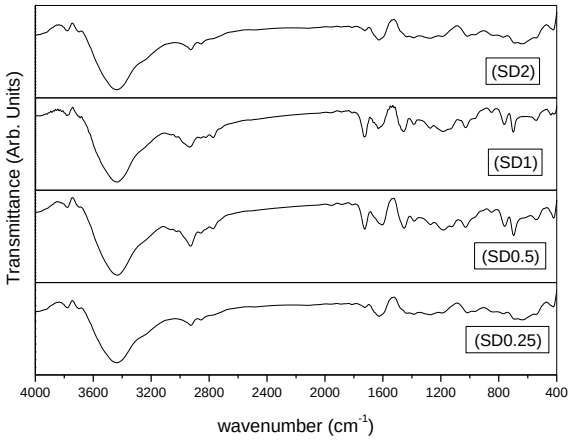


Fig. 1 FTIR of styrene-co-DMAEMA.

Fig. 2 shows the 1HNMR spectra of the copolymers. The measurements were conducted in deuterated chloroform. The protons signal of methyl group in (CH3-C) was observed at 1.2 ppm, whereas the protons signal of (CH2-C) were observed at 2.08 ppm. Sharp signal located at around 2.3 ppm is corresponding to methyl groups of (2CH3-N). The protons in (CH2-N) and (CH2-O) groups are appeared at 2.6 and 3.2 ppm, respectively. Signal of –CH2 of styrene is centred at 4.1 ppm. Finally, the signals at 7.08 and 6.7 ppm are attributed to the phenyl group and the –CH beside to phenyl group, respectively.

Fig. 3 (a-d) illustrates the FESEM images of the copolymers. From, Fig. 3 (a), it can be observed that the lowest concentration of DMAEMA has several holes representing a high degree of porosity. With increasing the concentration of DMAEMA, the porosity decreases. This means that by increasing DMAEMA concentration, the copolymer becomes more homogeneously distributed.

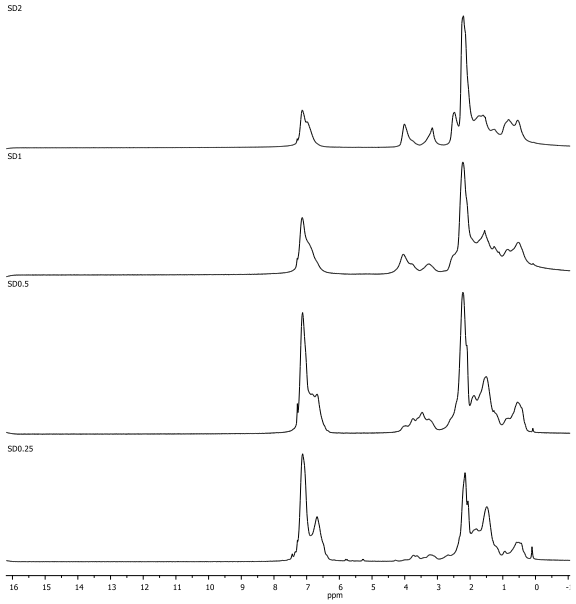


Fig. 2 [1]HNMR of styrene-co-DMAEMA.

3.2. Synthesis of cationic styrene-co-N,N-dimethylaminoethyl methacrylate

The cationic copolymers were synthesized as shown in Scheme 2. SD0.25, SD0.5, SD1, and SD2 were dissolved in CHCl3 and reacted with hexyl bromide at 60 °C, for 4 hours. The obtained products namely; CSD0.25, CSD0.5, CSD1, and CSD2, respectively; were washed several times with n-hexane for solidification. Cationic copolymers were characterized by FTIR, 1HNMR, WAXRD, TGA and FESEM.

Fig. 4 demonstrates the FTIR spectra of the cationic copolymers. The spectra show a vibrational band at 3027 cm-1 which is attributed to the stretching vibrations of unsaturated =CH stretching. The observed two bands at 2932 and 2859 cm-1 are corresponding to the asymmetric and symmetric stretching vibrations of saturated –CH. The two bands observed at 1725 and 1630 cm-1 is for ester group stretching and C=C vibrations of the aromatic ring, respectively.

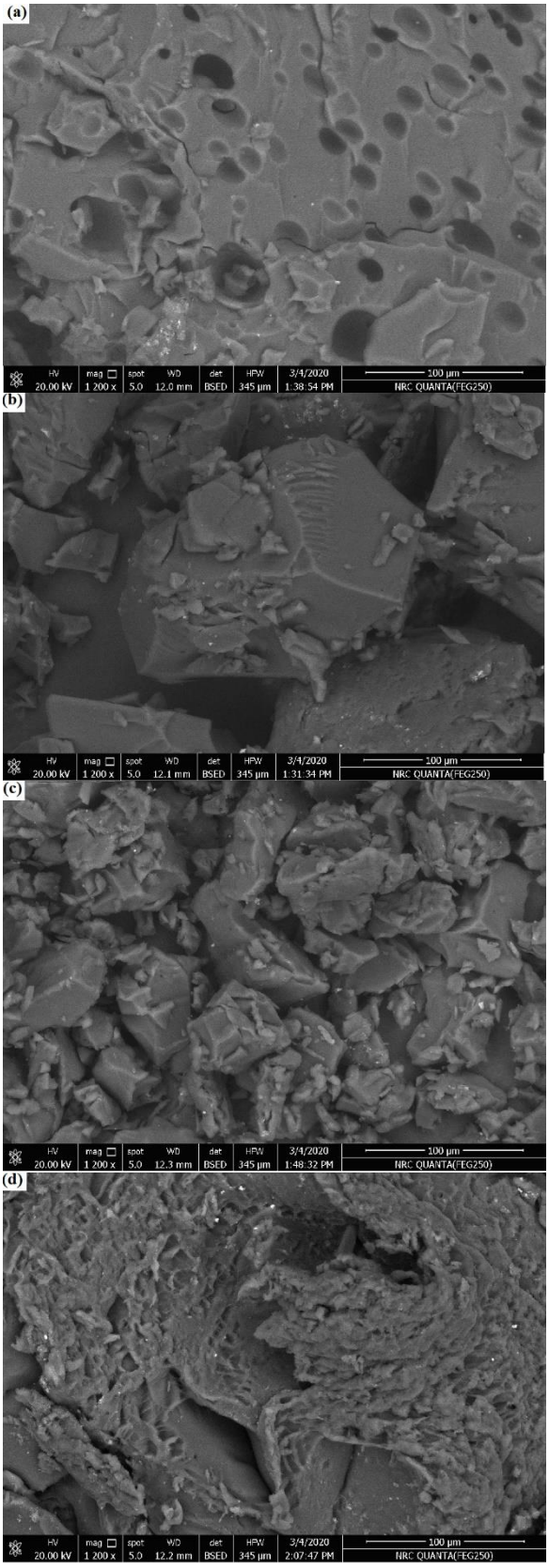
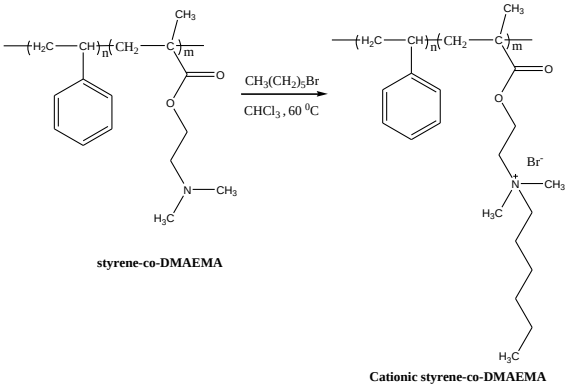


Fig. 3 FESEM of styrene-co-DMAEMA: (a) SD0.25, (b) SD0.5, (c) SD1 and (d) SD2.



Scheme 2: Synthesis of cationic styrene-co-DMAEMA.

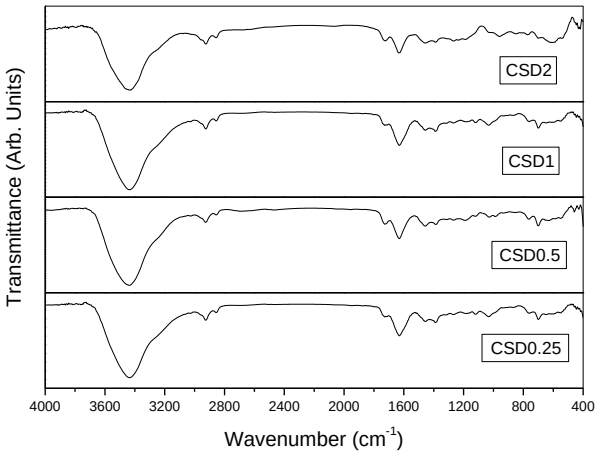


Fig. 4 FTIR of cationic styrene-co-DMAEMA.

[1]HNMR spectra of CSD1 in DMSO and CSD2 in D2O are depicted in Fig. 5. It can be observed that the signals appeared in 1HNMR of styrene-co-N,N-dimethylaminoethyl methacrylate are still present and there are two additional sharp signals appeared at 0.7 and 1.3 ppm. These bands are corresponding to CH3 and CH2 protons of hexyl group. These signals support the proposed molecular structure of the formed cationic styrene-co-DMAEMA.

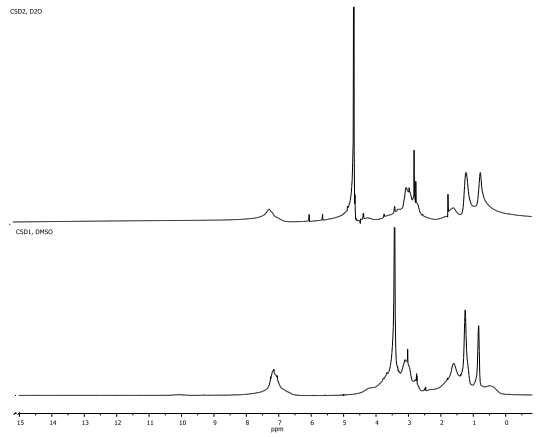


Fig. 5 1HNMR of cationic styrene-co-DMAEMA.

The WAXRD spectra of cationic styrene-co-DMAEMA are represented in Fig. 6. The absence of sharp diffraction lines and the presence of only broad humps are indicative of the amorphous structure of the prepared cationic styrene-co-DMAEMA.

Fig. 7 depicts the TGA curves of the cationic copolymers. For cationic copolymers CSD0.25 and CSD0.5, there is a slight influence on the weight loss temperatures. In case of CSD1 and CSD2, weight loss temperatures are shifted towards low temperatures. These behaviours may be attributed to the decrease in the aromatic part (styrene) concentration and increase in aliphatic part (cationic DMAEMA) concentration. The decomposition temperatures are represented in Table 1. The cationic copolymers decompose completely at about 500 °C.

Fig. 8 (a-d) shows the FESEM images of the cationic copolymers. There are some pores in the non-cationic copolymers. From the FESEM images, the pores are disappeared as a result of alkylation. Besides, the particle size is increased due to alkylation from CSD0.25 to CSD2.

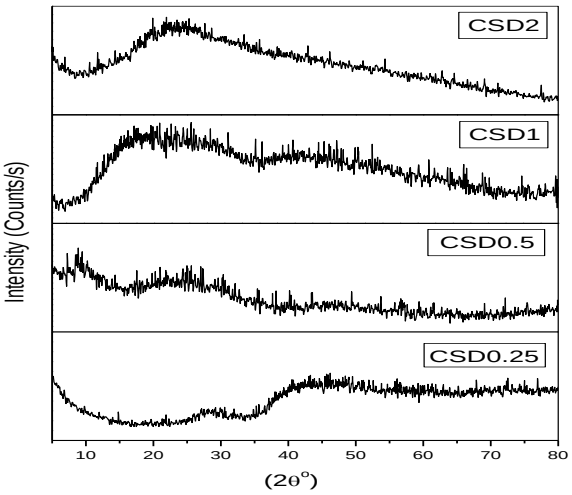


Fig. 6 WAXRD of cationic styrene-co-DMAEMA.

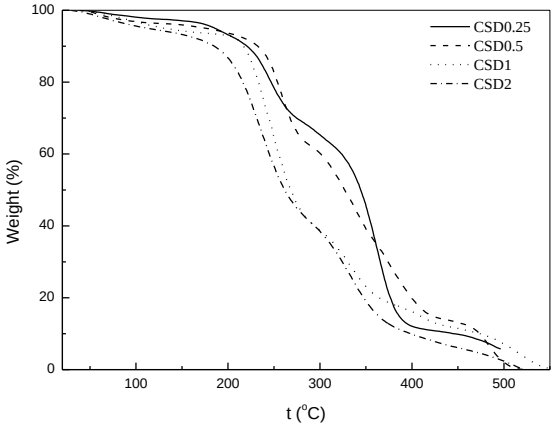


Fig. 7 TGA of cationic styrene-co-DMAEMA.

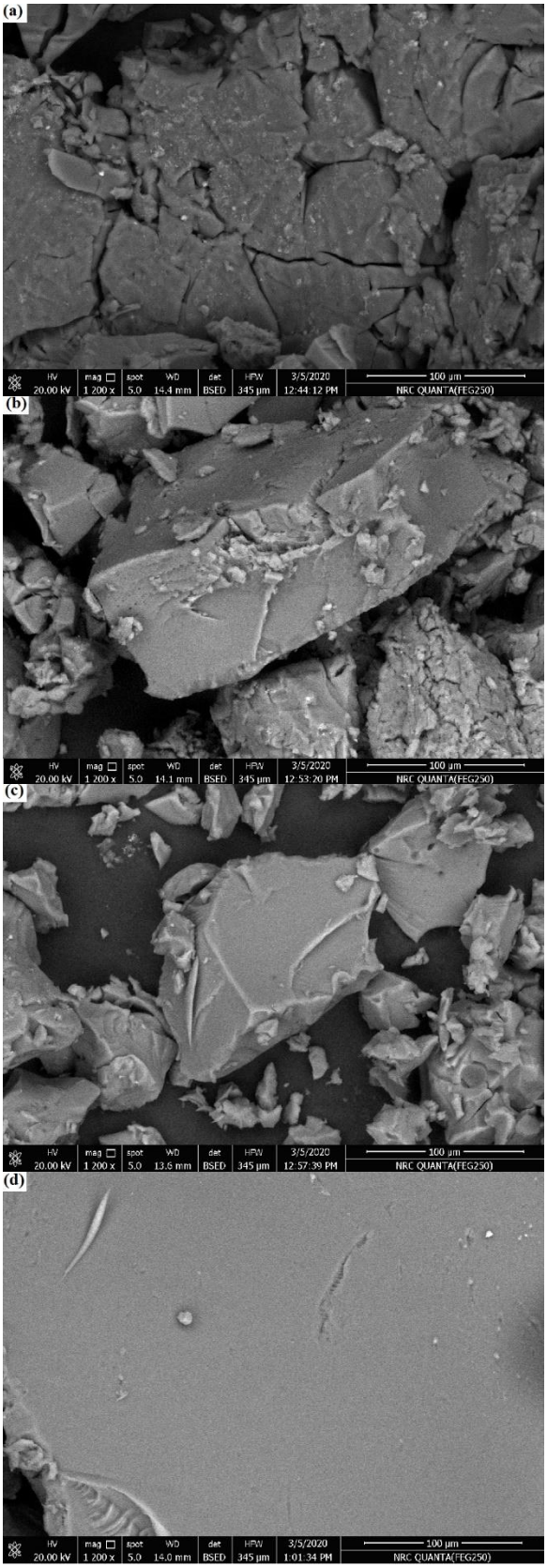


Fig. 8 FESEM of cationic styrene-co-DMAEMA: (a) CSD0.25, (b) CSD0.5, (c) CSD1, (d) CSD2.

Table 1 Weight loss temperatures of the cationic copolymers.

Code	T^a (°C)	T^b (°C)
CSD0.25	205-300	225-425
CSD0.5	205-305	395-495
CSD1	180-300	360-535
CSD2	185-290	355-540

Where T^a and T^b are the first and the second decomposition temperatures, respectively.

3.3. Biological activity against Escherichia coliO145 and Staphylococcus aureus bacteria

Table 2 lists the results of biological activity of the prepared non-cationic styrene-co-N,N-dimethylamino ethyl methacrylate; SD1, SD2 and the cationic copolymers; CSD0.5, and CSD1; against E.coli O145 and S. Aureus strains. It is obvious from the results that the copolymers SD1 and SD2 have the strongest antibacterial activity among the prepared non-cationic copolymers. They show also stronger activity against E. coliO145 bacterial strains than S. aureus strains. For the cationic copolymers, it is found that CSD0.5, and CSD1 have the strongest activity against both E.coliO145 and S. aureus strains. Images in Fig. 9 illustrate the inhibition zones of all the prepared cationic copolymers. It is observed that copolymers (CSD0.5, CSD1) have the largest inhibition zones and hence the strongest antibacterial activity against both bacteria. Fig. 10 shows the images of different concentrations of (CSD0.5, CSD1) copolymers and their inhibition zones against both E. coli O145 and S. aureus. It is clearly observed that cationic CSD0.5 and CSD1 copolymers have the strongest antibacterial activity than the non-cationic SD1, and SD2 copolymer.

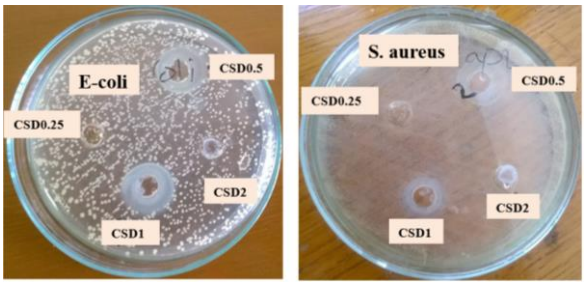


Fig. 9 Images of inhibition zones of cationic styrene-co-DMAEMA towards E. coli and S. aureus.

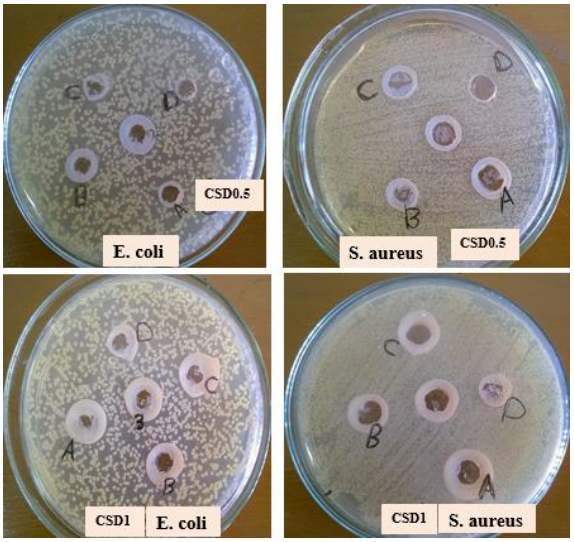


Fig. 10 Images of inhibition zones of different concentrations of CSD0.5 and CSD1 copolymers towards E. coli and S. aureus.

Table 2 Different concentrations of cationic and non-cationic copolymers and their inhibition zones toward E. coli and S. aureus strains.

code	Concentration(ug/ml)	Inhibition zone (mm)	code	Concentration(ug/ml)	Inhibition zone (mm)
E. coli O145			***S. aureus***		
CSD 0.5	10	14	CSD 0.5	10	12
	20	9		20	14
	30	12		30	11
	40	10		40	12
	50	8		50	9
CSD 1	10	14	CSD1	10	15
	20	15		20	17
	30	14		30	14
	40	16		40	16
	50	12		50	11
SD1	10	12	SD1	10	12
	20	11		20	12
	30	11		30	10
	40	11		40	0
	50	0		50	0
SD2	10	13	SD2	10	12
	20	13		20	0
	30	12		30	0
	40	0		40	0
	50	0		50	0

4. Conclusion

Copolymers of styrene and N,N-dimethylamino ethyl methacrylate were prepared and characterized using FTIR, 1HNMR, and FESEM. The reaction of the formed copolymers with hexyl bromide leads to the formation of the corresponding cationic copolymers which were characterized with different techniques. It was concluded that FTIR, and 1HNMR proved the structure of the formed copolymers. WAXRD proved the amorphous structure of the prepared copolymers. FESEM showed the surface morphology for the cationic and non-cationic copolymers. The shape of the surface of the non-cationic copolymers contains pores and by alkylation, the pores disappeared. Cationic copolymers showed the strongest antibacterial activity against both Escherichia coli O145 and Staphylococcus aureus isolates than the non-cationic ones.

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