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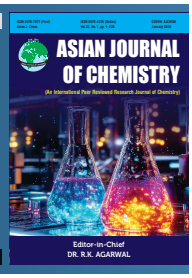

Design and Synthesis of Quaternized Antimicrobial Polymer against *Escherichia coli*

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New strategies and approaches are required due to the rising levels of antibiotic resistance, which are frequently brought on by long-term misuse or overuse of conventional antimicrobial medications. Due to their minimal potential to produce drug-resistant bacteria, effective antimicrobial polymers have been garnering more attention. The incorporation of quaternary ammonium groups into polymers is among the most beneficial methods for fabricating biomaterials with antimicrobial properties. This study presents the synthesis pathway on developing quaternized jeffamine polymer (QJP) with efficient antimicrobial property. The QJP was prepared by post polymerization modification of Jeffamine ED2003 and the structural integrity of the QJP was investigated by NMR and thermal properties. The synthesized QJP exhibited activity against *E. coli* and kill time assay revealed the synthesized polymer has a bacteriostatic effect.

Keywords: Jeffamine polymer, *Escherichia coli*, Minimum inhibitory concentration, Time kill assay.

INTRODUCTION

The struggle against harmful microbes in a variety of domains is an ongoing and routine effort for humans. Contamination by microorganisms is an extreme concern in several sectors, including implanted medical devices (ranging from catheters to artificial hips), infection caused by surgical devices, dental restoration, food packaging, storage, *etc*. [1-3]. Unfortunately, drug-resistant microbial strains are rapidly emerging and spreading around the world, making it harder to treat common infectious diseases. This has resulted in extended illness, disability and even death, as small molecular compounds are easily able to mutate and escape the specific targets of these antibiotics. Furthermore, small molecular antibiotics have limited effectiveness in certain applications and overuse of antibiotics has led to an increase in multi-drug resistant bacteria, posing a hazard to global healthcare systems in recent decades.

Novel approaches to eradicate and controlling these infections could significantly improve public health. Antimicrobial polymers are of special interest as novel classes of materials that can detect, mitigate, combat and/or lessen illnesses caused by bacteria, fungus, viruses or parasites [4-6]. Thus, it is not surprising that there has been a surge in interest in macromolecular (polymer) antibacterial drugs. In addition to polymers that have inherent antimicrobial action, macromolecular antimicrobial agents can also be polymers that have been combined with small antimicrobial agents [7,8].

There have been tremendous efforts made over the period of the last few decades to discover antimicrobial agents that are based on polymers that are even more effective. These efforts have been made either by attempting to synthesize new polymers with different structures, compositions or architectures, or by attempting to enhance the antimicrobial action of existing antibacterial polymers through the process of functionalization [7-11]. Piperazine attracts the spotlight since its derivatives have a plethora of significant pharmacological properties, including those of an antidepressant, antifungal, antimalarial, anticancer, anticonvulsant and anthelmintic [12,13]. Innumerable conventional medications that belong to different pharmacological classifications make considerable use of this heterocyclic compound [14].

Jeffamines are polyetheramines with primary amino end groups linked to the ends of a polyether backbone which is made of either ethylene oxide, propylene oxide or a combination of both [15]. The Jeffamine family consists of monoamines, diamines and triamines that depends on a main framework.

Functional groups in jeffamine can be altered using various antimicrobial agents. A wide range of works reported the utilization of jeffamine as biomaterials in biomedical aaplications [16]. These substances are hydrophilic in nature and are utilized as antistatic agents and epoxy modifiers. They offer versatility for applications such as water-based coatings, water-swellable polyamides and textile treatment. Due to its strong antimicrobial properties, it can also be employed in personal care products [17-19], thermoresponsive hydrogels and thermo-associative nanoparticles for drug delivery [20-22].

Based on these concepts, the interaction between piperazine and epichlorohydrin to synthesize a bifunctional coupler, featuring a chlorohydrin group on one end and an azetidinium group on the other was investigated [23]. Jeffamine, a polyetheramine consisting of two amino group at the terminals is reacted with maleic anhydride to produce jeffamine-*bis*-maleamic acid. Synthesized bifunctional coupler was reacted with jeffamine-*bis*-maleamic acid to produce quaternized jeffamine polymer.

EXPERIMENTAL

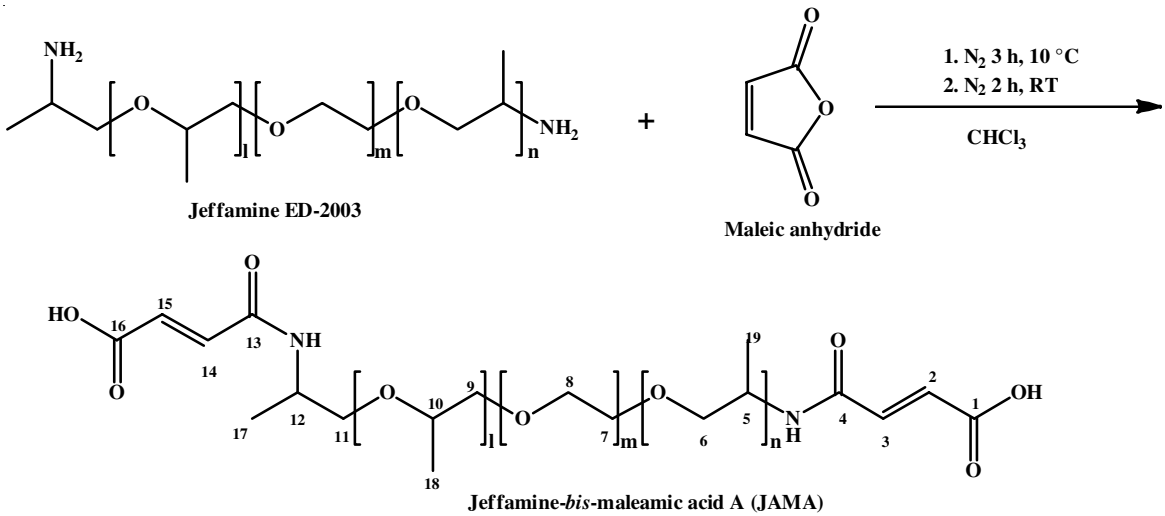
Maleic anhydride (≤ 99%, Sigma-Aldrich), piperazine (≤ 99%, Sigma-Aldrich), jeffamine ED-2003 (≤ 99%, Sigma-Aldrich), epichlorohydrin (≤ 99% , Merck) were employed without further purification. Jeffamine ED-2003 [O,O′-*bis*(2-aminopropyl)-polypropylene glycol-block-polyethylene glycol-block-polypropylene glycol] is a jeffamine ED-2003 is a blend of polyether diamines with an average molecular weight of 2000 g/mol, based on a primary PEG backbone. Dichloromethane, diethyl ether, hexane, toluene and dimethyl sulfoxide were procured from Merck, Germany and used as received.

Synthetic routes for quaternized jeffamine polymer (QJP)

Step 1: Synthesis of jeffamine-*bis*-maleamic acid A (JAMA): *bis*-Maleamic acid was prepared by the modification of jeffamine ED-2003 with maleic anhydride following a method reported in the literature [24,25]. Two equivalents of maleic anhydride (0.931 g, 0.0095 M) was dissolved in 15 mL of chloroform and kept under nitrogen atmosphere at 10 ºC. The one equivalent of jeffamine ED-2003 (9.5 g, 0.00475 M) was added dropwise over a period of 3 h and thereafter mixture was allowed to stir at room temperature for 2 h under nitrogen atmosphere. The chloroform was removed through the rotavapor and then dried to obtain yellow viscous jeffamine-*bis*-maleamic acid **A** (**Scheme-I**). Further jeffamine-*bis*-maleamic acid **A** was purified using toluene to remove unreacted reactants under vacuum. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 12 (m, ^{1,16}COOH), 8 (m, 1H, NH), 6.15-6.24 (m, 2H, ^{2,3,14,15}CH$_2$), 3.9 (m, 1H, ^{5,12}CH), 3.6 (m, 1H, ^{10}CH), 3.24-3.51 (m, 2H, ^{6,7,8,9,11}CH$_2$), 0.94-1.1 (m, 3H, ^{17,18,19}CH$_3$); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 166 (CONH^{4,13}), 165 (COOH^{1,16}), 133 (C^{2,15}), 132 (C^{3,14}), 74.6 (C^{10}), 69-73 (C^{6,7,8,9,11}), 45.8 (C^{5,12}), 16.9-17.9 (C^{17,18,19}).

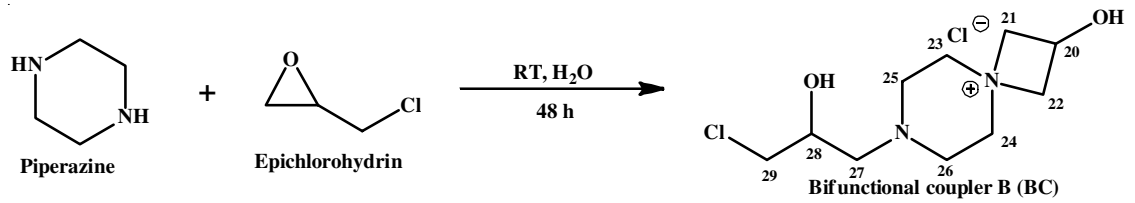
Step 2: Synthesis of bifunctional coupler B (BC): The bifunctional coupler was synthesized as reported elsewhere [23]. Two equivalents of epichlorohydrin (3 mL, 0.038 M) was added dropwise to the solution containing one equivalent of piperazine (1.65 g, 0.019 M) in 15 mL of water and the reaction mixture was stirred at 25 ºC for 48 h. Water was removed through the rotavapor to obtain a white solid bifunctional coupler **B** (**Scheme-II**). This compound was further purified *via* extracting the aqueous solution of the coupler with dichloromethane and then simply removed the water in vacuum [23,26,27]. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 6.5 and 5.3 (m, OH), 4.6 (m, 1H, ^{20}CH), 4.49 and 4.19 (m, 2H, ^{21,22}CH$_2$), 3.81 (m, 1H, ^{28}CH), 3.42-3.69 (m, 2H, ^{23,24,29}CH$_2$), 2.22-2.82 (m, 2H, ^{25,26,27}CH$_2$); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 70.73 (C^{21,22}), 68.2 (C^{28}), 61.28 and 60.11 (C^{23,24}), 59.2 (C^{27}), 58.28 (C^{20}), 48.86 (C^{29}), 48.38 and 47.91 (C^{25,26}) ppm.

Step 3: Synthesis of quaternized jeffamine polymer C (QJP): The bifunctional coupler **B** was reacted with jeffamine-*bis*-maleamic acid **A** for 24 h at 90 ºC in the presence of ethanol. After the completion of reaction, ethanol was removed through the rotavapor to obtain a thick yellow viscous liquid quaternized jeffamine polymer **C** (**Scheme-III**) and purified using hexane and diethyl ether. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 12 (m, ^{1,16}COOH), 6.39-6.48 (m, 2H, ^{2,3,14,15}CH$_2$), 6 (m, OH), 4.44 (m, 1H, ^{20}CH), 4-4.19 (m, 2H, ^{21,22}CH$_2$), 3.89 (m, 1H, ^{28}CH), 3.7 (m, 1H, ^{5,12}CH), 3.52 (m, 1H, ^{10}CH), 3.4-3.57 (m, 2H, ^{6,7,8,9,11}CH$_2$), 3.2-3.37 (m, 2H, ^{23,24,29}CH$_2$), 2.8-3.1 (m, 2H, ^{25,26,27}CH$_2$), 0.94-

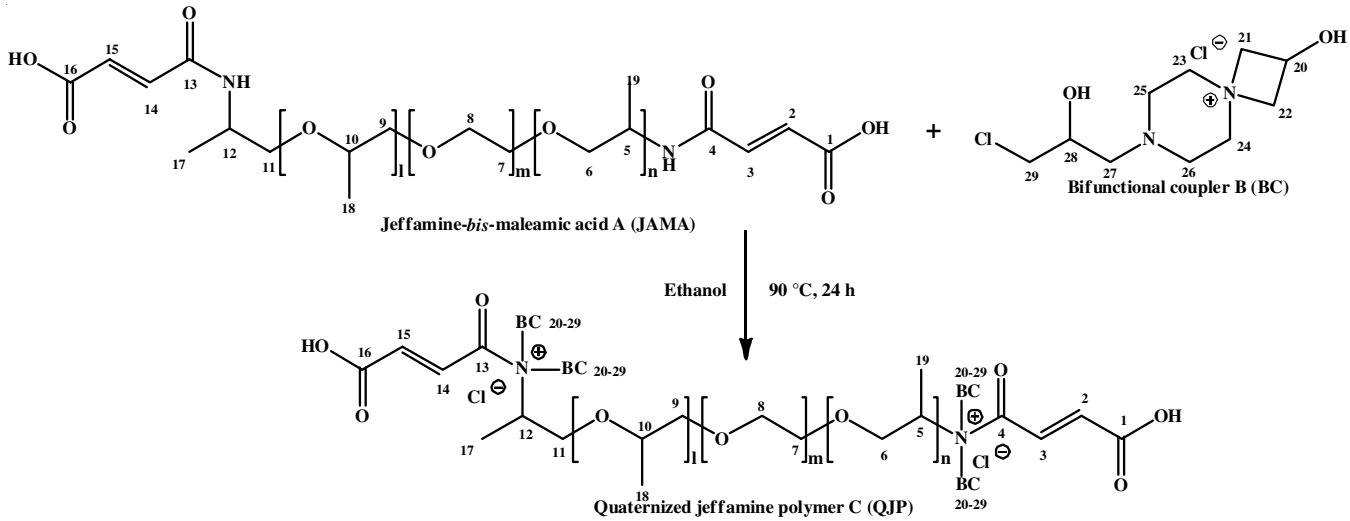


Jeffamine-*bis*-maleamic acid A (JAMA)

Scheme-I: Synthetic route of jeffamine-*bis*-maleamic acid **A** (JAMA)



Scheme-II: Synthetic route of bifunctional coupler **B** (BC)



Scheme-III: Synthetic route of quaternized jeffamine polymer **C** (QJP)

1.22 (m, 3H, $^{17,18,19}CH_3$); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 175 (CO), 173 (^{1,6}COOH), 133 ($C^{2,15}$), 131 ($C^{3,14}$), 74.5 (C^{10}), 69.7-70.6 ($C^{6,7,8,9,11}$), 64.2 ($C^{21,22}$), 61 (C^{28}), 59-60.4 ($C^{23,24,27}$), 56.3 (C^{20}), 50.2 ($C^{5,12}$), 47.7-48.5 ($C^{29,25,26}$), 14.6-18.7 ($C^{17,18,19}$).

Characterization: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance Neo 400 spectrometer (400 MHz), Switzerland. DMSO-d_6 were used as a solvent and tetramethylsilane as an internal standard. Thermal properties were determined using TGA and DSC. Both analyses were done using TA instrument SDT Q600 V20.9 over a temperature range of 10-600 ºC at a heating rate of 10 ºC/min in a nitrogen atmosphere using alumina pan.

***In vitro* antimicrobial screening:** The antimicrobial activity of the synthesized polymeric compound was screened against *Escherichia coli* (ATCC 25922). The bacterial culture was procured from the American Type Culture Collection (ATCC) Manassas, Virginia, USA and maintained as per standard protocol.

Agar diffusion method: The antimicrobial activity of the quaternized jeffamine polymeric (QJP) compound was determined by employing agar well diffusion method against *E. coli* bacterial strain. The nutrient agar media was prepared, autoclaved and about 20 mL of agar media was dispensed into petri dish. It was allowed to solidify for 15 min inside laminar air flow chamber. A subculture of selected bacterial strain at a volume of 200 µL, equivalent to 10^6 CFU/mL, was uniformly spread onto the surface of a petri dish containing 20 mL of nutrient agar, using a sterile cotton swab and wells were punched using a sterile gel borer. Five wells, each measuring eight millimetres in diameter, were made on the agar for the bacterial strain. The working solutions of three different concentration 5 mg/mL, 10 mg/mL and 20 mg/mL was dissolved in DMSO. The negative control well was loaded with 100 mL of DMSO, the positive control well with 100 µL of antibiotic ampicillin at 0.1 mg/mL, and the remaining three wells with 100 µL of polymeric compound at 5, 10, and 20 mg/mL concentrations. Afterward, the petri dish was incubated at 37 ºC for a duration of 24 h to observe zone of inhibition surrounding the well in mm.

Minimum inhibitory concentration: The quaternized jeffamine polymer (QJP) sample was dissolved in DMSO at different concentrations (2.5, 1.25, 0.625, 0.3125 mg/mL). Further, 50 µL of each concentration ranging from 2.5-0.3125 mg/mL (2.5, 1.25, 0.625, 0.3125 mg/mL) was distributed in 96 well plate to the different wells; followed by the addition of 100 µL of nutrient broth and 50 µL of microbial culture at concentration of 10×10^5 colony forming units (CFU)/mL (total volume of 200 µL). UV spectrophotometer (ELX-800, BioTek, USA) with 600 nm wavelength was used to study the antimicrobial activity of the QJP sample along with ampicillin (0.1 mg/mL) as positive control and DMSO served as negative control. The optical density (OD) was measured at 600 nm at 0 h, incubated overnight at 37 ºC and measured again after 24 h. Then the percentage inhibition in microbial growth was calculated in comparison with control using the following formula:

$$\text{Inhibition (\%)} = 100 - \left(\frac{OD_i}{OD_f}\right) \times 100$$

where OD_i is the difference between initial (before incubation) and final (after incubation) OD of QJP; OD_f is the difference between OD of control.

Time kill assay: The time-kill assay was studied at the concentrations ranging from 2.5 to 0.078 mg/mL (2.5, 1.25, 0.625, 0.3125, 0.156, 0.078 mg/mL). The time-kill assay for the polymer was investigated against *E. coli* using the micro-broth dilution technique. A similar method to the MIC technique was adopted and continuous incubation at 37 ºC was used to measure the growth of microorganisms at 600 nm for 0, 4, 8, 12, 16, 20, 24 h. For every sample concentration, three duplicates were obtained and the mean absorbance was reported. The percentage reduction was computed using the initial (before to incubation) and final (after incubation) optical densities at 600 nm. Ampicillin an antibacterial standard drug was selected at 0.1 mg/mL.

RESULTS AND DISCUSSION

NMR studies of jeffamine-*bis*-maleamic acid A (JAMA): ^{1}H NMR spectrum of JAMA **A** (Fig. 1a) shows peaks for carboxylic acid at δ 12 (m, ^{1,16}COOH) ppm. The signal at δ 8 (m, 1H, NH) ppm attributed to NH protons of jeffamine ED-2003. The signals of unsaturated bonds were observed from δ 6.15-6.24 (m, 2H, $^{2,3,14,15}CH_2$) ppm. The signals at δ 3.9 (m, 1H, ^{5,12}CH) and 3.6 (m, 1H, ^{10}CH) ppm corresponds to CH protons adjacent to amine and methyl group, respectively. The CH_2 protons of polyether chain was observed from δ 3.24-3.51 (m, 2H, $^{6,7,8,9,11}CH_2$) ppm. The methyl protons corresponding to polyether chain of jeffamine ED-2003 attributed from δ 0.94-1.1 (m, 3H, $^{17,18,19}CH_3$) [24,25,28,29].

^{13}C NMR spectrum of JAMA **A** (Fig. 1b) shows carbonyl amide and carboxylic acid signal at δ 165 (CONH) and 166 (COOH) ppm. The signals at δ 133 ($C^{2,15}$) and 132 ($C^{3,14}$) ppm attributed to unsaturated CH_2 carbon atoms. The signals at δ 74.6 (C^{10}) and 45.8 ($C^{5,12}$) ppm assigned to CH carbon atom of methyl and amine group environment. The CH_2 carbon signal of polyether chain were observed from δ 69-73 ($C^{6,7,8,9,11}$) ppm. The CH_3 carbon atoms of jeffamine-ED 2003 were seen from δ = 16.9-17.9 ($C^{17,18,19}$) ppm [24,25,28,29].

NMR studies of bifunctional coupler B (BC): ^{1}H NMR spectrum of BC **B** (Fig. 2a) shows peaks for the azetidinium ring protons at δ 4.19-4.6 ppm (m, 1H, ^{20}CH and m, 2H, $^{21,22}CH_2$). The peaks of the chlorohydrin groups were found at δ 3.81 (m, 1H, ^{28}CH), 3.48 (m, 2H, $^{29}CH_2$) and 2.39 (m, 2H, $^{27}CH_2$) ppm. Protons corresponding to the piperazine group were split into two regions, that is, protons adjacent to chlorohydrin group at δ 2.55-2.76 (m, 2H, $^{25,26}CH_2$) ppm and the protons adjacent to azetidinium group at 3.49 (m, 2H, $^{23,24}CH_2$) ppm. Peaks for the alcohol groups were found at δ 6.5 ppm and δ 5.3 ppm, which are associated with the azetidinium and chlorohydrin groups, respectively [23].

^{13}C NMR spectrum of BC **B** (Fig. 2b) shows carbon signal at δ 70.73 ($C^{21,22}$) and 58.28 (C^{20}) ppm associated with the azetidinium ring. The peaks of the carbon atoms which is associated with the chlorohydrin group were observed at δ 68.2 (C^{28}), 59.2 (C^{27}) and 48.86 (C^{29}) ppm. The signals of carbon atoms corresponding to the piperazine group were found at δ 61.28 and 60.11 ($C^{23,24}$), 48.38 and 47.91 ($C^{25,26}$) ppm [23].

NMR studies of quaternized jeffamine polymer C (QJP): ^{1}H NMR spectrum of QJP **C** (Fig. 3a) shows peaks for carboxylic acid at δ 12 (m, ^{1,16}COOH) ppm. The signals of unsaturated bonds were observed from δ 6.15-6.24 (m, 2H, $^{2,3,14,15}CH_2$) ppm. The signals for the alcohol groups corresponding to azetidinium and chlorohydrin group were observed at δ 6.0 (m, OH) ppm. The azetidinium ring protons associated with bifunctional coupler were observed at δ 4.44 (m, 1H, ^{20}CH) and 4.0-4.19 (m, 2H, $^{21,22}CH_2$) ppm. The CH proton peak adjacent to hydroxyl

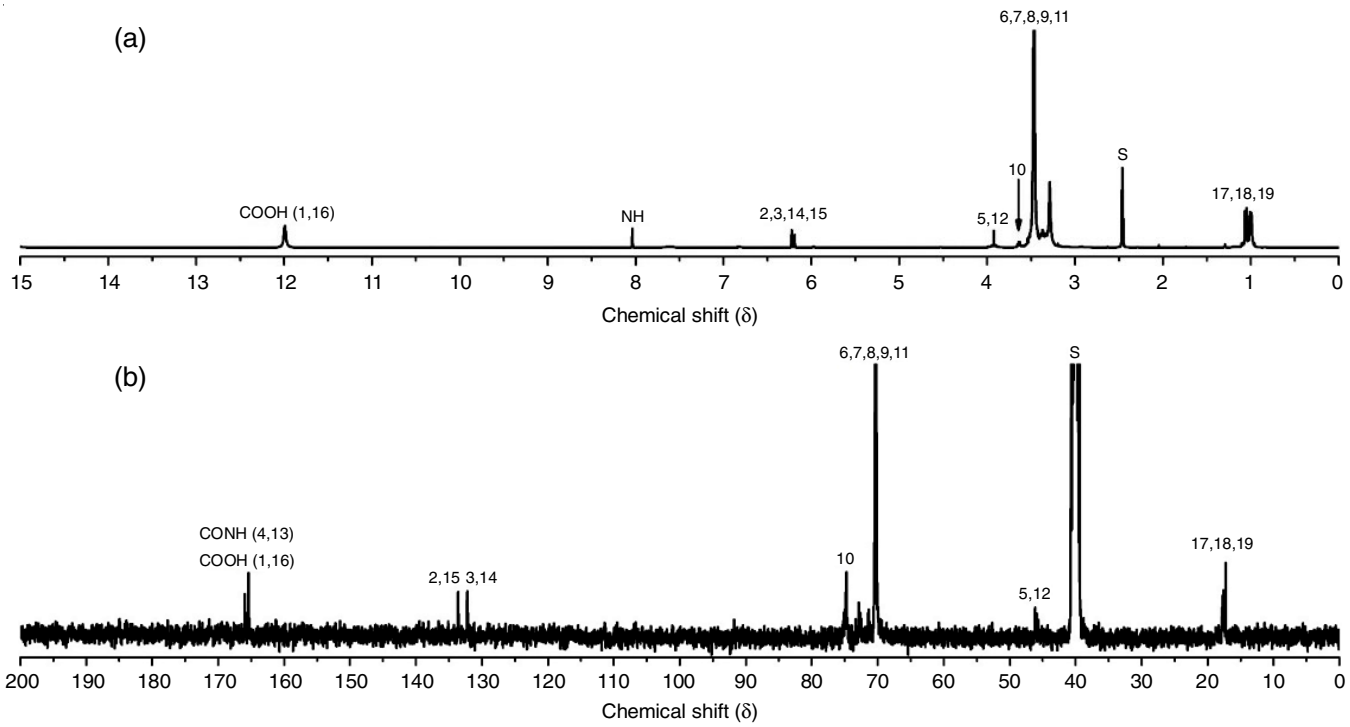


Fig. 1. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of jeffamine-*bis*-maleamic acid **A** (JAMA) (Solvent: DMSO-d_6)

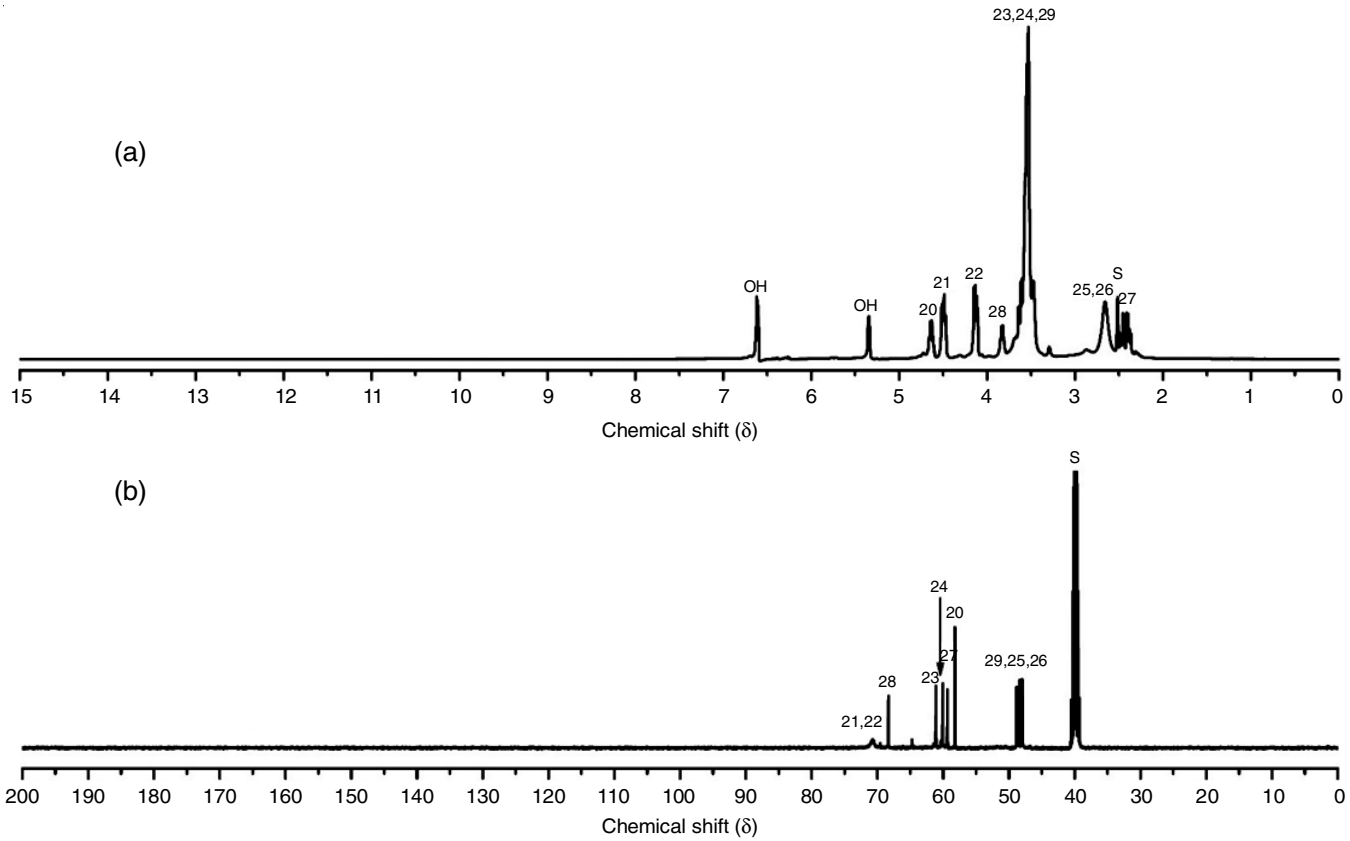


Fig. 2. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of bifunctional coupler **B** (BC) (Solvent: DMSO-d_6)

group observed at δ 3.89 (m, 1H, ^{28}CH) ppm. The signals at δ 3.7 (m, 1H, ^{5,12}CH) and 3.52 (m, 1H, ^{10}CH) ppm attributed to CH protons corresponding to amine and methyl groups of polyether chain, respectively. The CH_2 protons of polyether chain was observed from δ 3.4-3.57 (m, 2H, $^{6,7,8,9,11}CH_2$) ppm. The signals for CH_2 protons corresponding to quaternary amines were observed at 3.2-3.37 (m, 2H, $^{23,24,29}CH_2$) ppm. The peaks for CH_2 protons attributed to tertiary amine were observed at δ 2.8-3.1 (m, 2H, $^{25,26,27}CH_2$) ppm. The methyl protons corresponding to polyether chain of jeffamine ED-2003 attributed from δ 0.94-1.22 (m, 3H, $^{17,18,19}CH_3$) ppm. The major observation is absence of NH proton confirms the formation of QJP molecule.

^{13}C NMR spectrum of QJP (Fig. 3b) shows carbonyl carbon atom and carboxylic acid signal at δ 175 (CO) and 173 (COOH) ppm. The peaks at δ 133 ($C^{2,15}$) and 132 ($C^{3,14}$) ppm ascribed to unsaturated CH_2 carbon atoms. The signals at δ 74.5 (C^{10}) and 50.2 ($C^{5,12}$) ppm assigned to CH carbon atom of methyl and amine group, respectively. The CH_2 carbon peaks of polyether chain were observed from δ 69.7-70.6 ($C^{6,7,8,9,11}$) ppm. The CH_2 carbon peaks corresponding to azetidinium group were attributed at δ 64.2 ($C^{21,22}$) ppm. The CH carbon signals corresponding to hydroxyl group of chlorohydrin and azetidinium were observed at δ 61 (C^{28}) and 56.3 (C^{20}) ppm, respectively. The signals from δ 59-60.4 ($C^{23,24,27}$) and 47.7-48.5 ($C^{29,25,26}$) ppm attributed to CH_2 carbon atom corresponding to piperazine and chlorohydrin groups, respectively. The methyl carbon atoms of jeffamine-ED 2003 were observed from δ 14.6-18.7 ($C^{17,18,19}$) ppm.

Thermal analyses

Differential scanning calorimeter (DSC): The thermal transition of quaternized jeffamine polymer C (QJP) was studied by differential scanning calorimeter. The glass transition temperature (T_g) of the Jeffamine Ed-2003 was observed to be 105 ºC (Fig. 4a). The QJP exhibited multiple exothermic and endothermic peaks.

Thermal gravimetric analysis (TGA): Thermal stability of quaternized jeffamine polymer (QJP) was studied using TGA (Fig. 4b). Two phases of weight loss can be observed from 88.06-123.69 ºC, 218.56-357.7 ºC and 448.7-540.8 ºC, respectively (Table-1). The major weight losses were observed between 218.56-357.7 ºC and 448.7-540.8 ºC. The methylene molecule linked to jeffamine ED-2003, maleic anhydride and bifunctional coupler showed weight loss of 7% from 357-448 ºC [30-32]. The maximum rate of weight loss was observed at temperature from 218.56-357.7 ºC (69.07%) is for amine groups of QJP and from 448.7-540.8 ºC (18.26%) is for irrespective of the

TABLE-1
THERMAL PROPERTIES OF
QUATERNIZED JEFFAMINE POLYMER (QJP)

Major weight loss transition (°C)	Rate of mass loss (%)	Residual weight % at 540.8 °C	T_{50} (°C)
88.06-123.69	3.09	0.28	320.5
218.56-357.7	69.07		
448.7-540.8	18.26		

T_{50} = Temperature at which 50% of weight loss occurred in TGA.

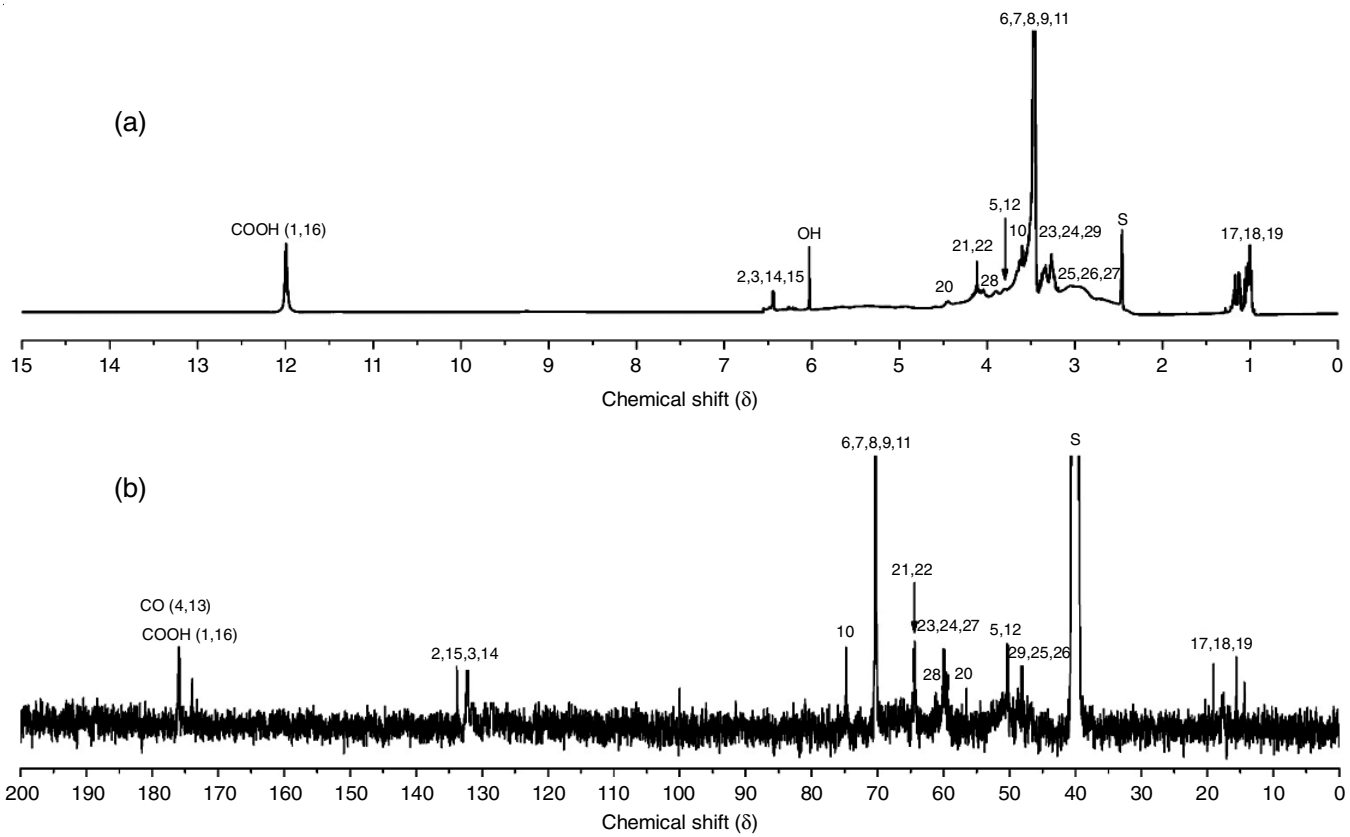


Fig. 3. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of quaternized jeffamine polymer **C** (QJP) (Solvent: DMSO-d_6)

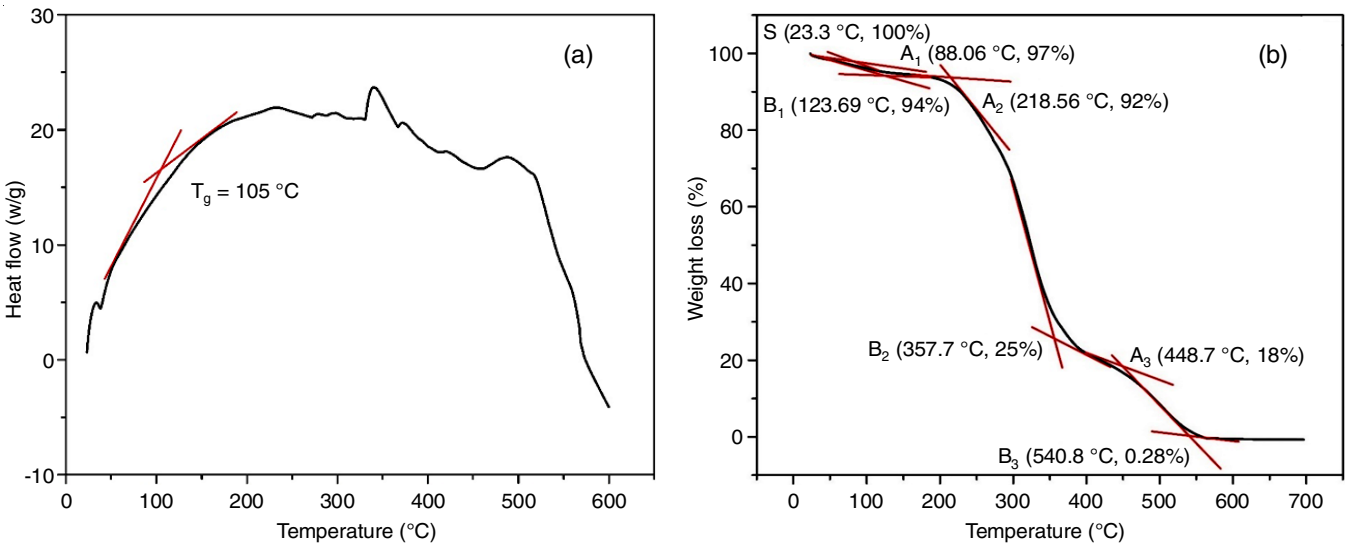


Fig. 4. (a) DSC and (b) TGA thermogram of quaternized jeffamine polymer (QJP)

polymers chemical structure [33,34]. The temperature at which 50% of weight loss occurred was 320.5 ºC. The polymer degraded completely at 542 ºC. Slight shift in the degradation temperature was observed in QJP in comparison with the standard jeffamine ED-2003.

$$\text{Rate of mass loss, } ML_1 = \left(\frac{m_{A1} - m_{B1}}{m_{A1}}\right) \times 100$$

$$\text{Rate of mass loss, } ML_2 = \left(\frac{m_{A2} - m_{B2}}{m_{A1}}\right) \times 100$$

$$\text{Rate of mass loss, } ML_3 = \left(\frac{m_{A3} - m_{B3}}{m_{A1}}\right) \times 100$$

where, m_{A1}, m_{A2}, m_{A3}, m_{B1}, m_{B2}, m_{B3} corresponds to mass at points A_1, A_2, A_3, B_1, B_2, B_3.

In vitro antimicrobial screening

Agar well diffusion method: Quaternized jeffamine polymer (QJP) showed significant zone of inhibition against pathogenic *E. coli* bacterial strain. At low concentration (5 mg/mL), QJP showed 17.1 mm of diameter *E. coli*. At 10 mg/mL

concentration, synthesized polymeric material showed 18.3 mm of diameter for *E. coli*. As the concentration was increased to 20 mg/mL, the polymeric material exhibited a considerably bigger zones of 19.2 mm (*E. coli*) diameter (Table-2). Increase in the zone of inhibition was observed, as the concentration of quaternized jeffamine polymer (QJP) increased. The efficient activity was due to the existence of positively-charged quaternary ammonium group, where the electrostatic interaction takes place between a negatively charged microbial cell wall and positively charged quaternary ammonium group that eventually leads to cell lysis [35-39]. In addition, the polymer also contains, –OH groups and counter ion (Cl^-), which are known for targeting cytoplasmic membrane of microorganisms. This leads to leakage in intercellular components followed by the death of microbes [40-43]. Due to these factors, QJP showed excellent antimicrobial activity.

TABLE-2
ZONE OF INHIBITION OF
QUATERNIZED JEFFAMINE POLYMER (QJP)

Polymer	Zone of inhibition (mm)
	E. coli
QJP (5 mg/mL)	17.33 ± 0.16
QJP (10 mg/mL)	18.56 ± 0.20
QJP (20 mg/mL)	19.53 ± 0.28
Ampicillin (0.1 mg/mL) (standard)	31.16 ± 0.20

Minimum inhibitory concentration (MIC): The synthesized quaternized jeffamine polymeric (QJP) molecule has demonstrated substantial effectiveness against *E. coli*, with a reported 50% inhibition generated by QJP against this bacterium. The 50% of inhibition against *E. coli* was observed at 0.3125 mg/mL,which is compared to standard antibacterial drug ampicillin (0.1 mg/mL). The percent inhibition for *E. coli* was examined in increasing concentration QJP (0.312-2.5 mg/mL). At lower concentration of the QJP (0.3125 mg/mL), the percent inhibition for *E. coli* was observed to be 51%. Gradual increase in the percent inhibition was observed as the concentration increased from 0.312-2.5 mg/mL. At a dosage of 2.5 mg/mL, a significant percent inhibition of *E. coli* (82%) was achieved. The polymer showed good inhibition against *E. coli* due to the presence of quaternary ammonium groups, hydroxy groups and counter ions. The obtained QJP results showed significant activity compared to the other reported polymer molecules [27,44-46].

Time kill assay: The time kill assay delivers more precise information about the effect of the test polymer on bacteria since the measurements take at different time intervals. Furthermore, this method has the ability to distinguish bactericidal activity from bacterial regrowth. Time kill curve (Fig. 5) is produced by plotting the log 10 of colony forming units per mL against time (0, 4, 8, 12, 16, 20 and 24 h) for *E. coli.* Ampicillin was employed as a positive control and DMSO solvent as a negative control. The investigation revealed QJP has a bacteriostatic effect on *E. coli* [47]. The growth curve of *E. coli* was treated with different concentrations (2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg/mL) of QJP demonstrated that polymeric compound could inhibit the growth and reproduction of *E. coli*.

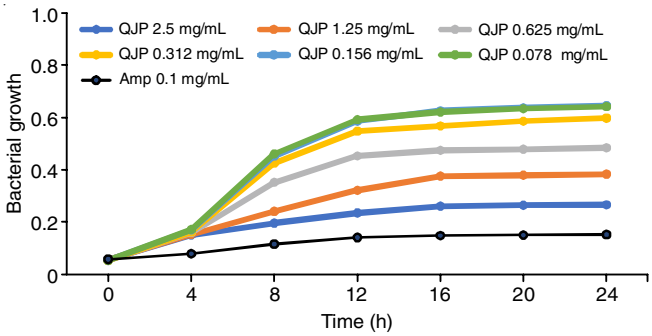


Fig. 5. Time kill assay of QJP at varying concentrations against *E. coli.* Ampicillin was taken as antibacterial standard

Increase in OD was observed at 4 h of inoculation and a significant increase at 8 h. As the time increased from 8 h to 24 h, no significant change in OD was observed. This demonstrates that the QJP has a bacteriostatic effect on *E. coli* [48,49].

Conclusion

Innovative polymer-based materials with antimicrobial properties are gaining attention as a promising solution to combat infections. In this study, the synthetic pathway on developing quaternized jeffamine polymer (QJP) bearing efficient functionalities (quaternary ammonium groups, counter ions (Cl^-) and hydroxy groups), which are responsible for exhibiting efficient antimicrobial activity against Gram-negative bacterium *E. coli* with an MIC of 0.3125 mg/mL and time kill assay revealed the synthesized polymer has a bacteriostatic effect.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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