Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.20267

64 (2017) 101–108
February

Synthesis of halogenated nanodendrimer as novel antimicrobial agents in water treatment

Afshin Maleki[a,*], Behzad Shahmoradi[a], Bagher Hayati[a], Mohammad Ahmadi Jebelli[a], Hiua Daraei[a], Sang W. Joo[b]

[a]*Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran, Tel. +988733626969; Fax: +988733625131; emails: maleki43@yahoo.com (A. Maleki), bshahmorady@gmail.com (B. Shahmoradi), bagher.hayati90@gmail.com (B. Hayati), m.ahmadijebelly@gmail.com (M.A. Jebeli), hiua.daraei@gmail.com (H. Daraei)*
[b]*School of Mechanical Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea, Tel. +82-53-810-2460; Fax: +82-53-810-4627; email: swjoo1@gmail.com*

Received 26 April 2016; Accepted 16 October 2016

ABSTRACT

Microbiological quality of drinking water is an important aspect of water quality. The aim of this research work was to fabricate and modify G2 and G4 dendrimers as novel antibacterial agent for local application. Poly(amidoamine) (PAMAM) (G_2 and G_4) dendrimers were fabricated and modified into quaternary ammonium salts using halogens groups (Cl, Br, I), and characterized by Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), and dynamic light scattering (DLS) analysis. The results of these analysis proved that nanostructure dendrimer and their quaternary ammonium salts are well fabricated. For evaluation of the antimicrobial property, the water samples were collected from rural drinking water resources, and the bacteria isolated and identified from these samples were *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*. The antimicrobial activities of PAMAM dendrimers (G_2 and G_4) and modified PAMAM dendrimers (G_2Cl, G_2Br, G_2I, G_4Cl, G_4Br, and G_4I) against gram-positive and gram-negative bacteria were examined by calculating minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and disc diffusion methods. Quaternary ammonium salts exhibited more antimicrobial efficacy against bacteria compared with unmodified ones. The most antibacterial effect was obtained by G_4I with MIC: 52, 50, 55, and 57 μg/ml, and MBC: 64, 67, 71, and 75 μg/ml for *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella oxytoca*, and *Escherichia coli*, respectively. The disc diffusion test of G_4I (60 μM) on different bacteria showed inhibition zone diameters of 31, 28, 26, and 25 ml for *Staphylococcus aureus, Bacillus subtilis, Klebsiella oxytoca*, and *Escherichia coli*, respectively.

Keywords: PAMAM; Antimicrobial agent; Microorganism; Dendrimer; Water

1. Introduction

Bacterial infections are the main factor threatening human health in various environments [1]. Therefore, antimicrobial agents are widely used in all activities including water purification to meet the required quality of products and thereby protect the safety of consumers. However, these compounds have some drawbacks. Toxicity to the environment, requiring high doses for effectiveness, and the short-term antimicrobial ability are the examples of their disadvantages [2,3]. Chemical disinfectants such as chlorine compounds and ozone have long been used to control variety of water-borne diseases [4–6]. They are not reliable due to production of dangerous disinfection by-products, and there are still great concerns in this respect [7]. Hence, there is an urgent need to explore innovative approaches that can enhance the safety of disinfection.

* Corresponding author.

One of the promising candidates is hyper-branched (HB) polymers, which are highly regarded today for numerous applications [8,9]. Kanai et al. [10] synthesized poly(cyanurateamine) and poly(triacrylatetrimine) HB polymers and evaluated their antibacterial properties against gram-positive and gram-negative bacteria. Dendrimers are HB polymers having unique characteristics affected by highly branched structure, multiple surface functional groups, and the vacant intra-branches spaces. Guest molecules could be absorbed and encapsulated by these vacant spaces. Meanwhile, functionalization of dendrimers can create a new and unique features in them [11]. For example, functionalization of surface groups of dendrimers with antimicrobial agents will create antimicrobial properties and can be applied to improve the efficiency, offer a longer lifetime, and minimize the toxicity of some antimicrobial agents [2,3]. Ahmadi Jebelli et al. [12] showed that poly(propylene imine) (PPI-G2) dendrimer possesses high antibacterial activity against gram-positive bacteria compared with gram-negative ones. Antimicrobial property of polyamidoamine (PAMAM) dendrimers reported by Lopez et al. [13] indicated their ability in deactivating various microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Chen et al. [14] synthesized quaternary ammonium functionalized poly(propylene imine) dendrimers and assessed their antibacterial activities using a bioluminescence method. They showed quaternary ammonium dendrimers are very strong biocides with different antibacterial properties, which is related to their size, the length of hydrophobic chains in the quaternary ammonium groups, and the counter anion [14]. Although influence of quaternary ammonium compounds in microbial destruction has been reported [12], the authors' literature review revealed that PAMAM dendrimer with halogen elements is not known for these specific antibacterial properties. Therefore, in this study, PAMAM dendrimers terminated with amine group were prepared and modified with halogen elements, and their antibacterial properties were evaluated in the removal of gram-positive and gram-negative bacteria from aqueous solution.

2. Materials and methods

2.1. Materials

CH_3I, $NH_4^+Cl^-$, dimethyl alkyl ammonium bromide, citric acid (CA), glutaric acid (GA), brain–heart infusion (BHI) nutrient broth, Mueller–Hinton agar, and sodium hypophosphite (SHP) were purchased from Merck (Germany).

2.2. Synthesis of modified dendrimers

PAMAM dendrimer was synthesized using divergent method by repeating the Michael addition of amino groups with methyl acrylate, followed by amidation of the resulting esters with excess ethylenediamine. The crude product was then transferred to a dialysis bag (MWCO5000; Sigma-Aldrich, France) for purification in deionized water for 48 h to remove unnecessary by-products (Table 1 and Fig. 1).

In the first phase, 20 g of soluble dendrimers (6.14 mmol of PAMAM-G_2 and 1.4 mmol of PAMAM-G_4) was mixed with 3.94 g (27.7 mmol) of CH_3I and 1.56 g (27.8 mmol) of KOH at 25°C for 2 h. KI, as a by-product, was isolated by filtration. In the next phase, 1.97 g (13.6 mmol) of ammonium iodide was added to the solution at 35°C for 2 h, and G_2I and G_4I were synthesized. Using the same methods, 1.76 g (13.9 mmol) of benzylchloride and 1.51 g (7 mmol) of dibromobutane were added to the solution in the second phase for the synthesis of G_2Cl, G_4Cl, G_2Br, and G_4Br, respectively [15–18].

Table 1
Properties of PAMAM and modified PAMAM

Symbol	Dendrimer	Molecular formula	Total number of amine groups	Number of amine end groups
A	PAMAM-G_2	$C_{166}H_{60}N_{54}O_{28}$	60	16
B	PAMAM-G_4	$C_{622}H_{1248}N_{250}O_{124}$	250	64
C	PAMAM-G_2Cl	$C_{166}H_{28}N_{54}O_{28}Cl_{16}$	60	16
D	PAMAM-G_2Br	$C_{166}H_{28}N_{54}O_{28}Br_{16}$	60	16
E	PAMAM-G_2I	$C_{166}H_{28}N_{54}O_{28}I_{16}$	60	16
F	PAMAM-G_4Cl	$C_{622}H_{1120}N_{250}O_{124}Cl_{64}$	250	64
G	PAMAM-G_4Br	$C_{622}H_{1120}N_{250}O_{124}Br_{64}$	250	64
H	PAMAM-G_4I	$C_{622}H_{1120}N_{250}O_{124}I_{64}$	250	64

2.3. Characterization of modified dendrimers

The spectroscopic characterization data of PAMAM and modified PAMAM dendrimers were reported by the following equipment. C-13 nuclear magnetic resonance (C-13 NMR) spectra of the dendrimers was recorded with a spectrometer (400 MHz, Bruker, Germany) using deuterated chloroform as solvent. Melting points were measured with a Mel-Temp melting point apparatus at a heating rate of 5°C/min. Fourier transform infrared (FTIR) spectra of dendrimers and quaternary ammonium dendritic copolymer networks were recorded on a spectrometer (Tensor 29, Bruker, Germany) in the region of 400–4,000 cm^{-1}. FTIR spectra were measured on KBr pellets prepared by pressing mixtures of 1 mg dried powdered sample and 100 mg spectrometry grade KBr under vacuum. Dynamic light scattering (DLS) analysis was performed on diluted (1:10) solutions upon double filtration by means of a 450-nm pore filter. DLS was measured at 25°C on a Spectra Physics 2050 light-scattering spectrophotometer at 363.5 nm wavelength of an argon laser. The spectrophotometer was equipped with a BI-200 goniometer and a BI-2030 correlator (Brookhaven). The DLS facilities were provided by Ausimont-Bollate (Milan, Italy).

2.4. Measuring of MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured with the method recommended by Clinical and Laboratory Standards Institute (CLSI) [19]. Tubes containing 10 ml of nutrient broth consisting of 10^8 CFU/ml of bacteria, and different dilutions of dendrimers were incubated at 37°C for 24 h. Each experiment was repeated three times.

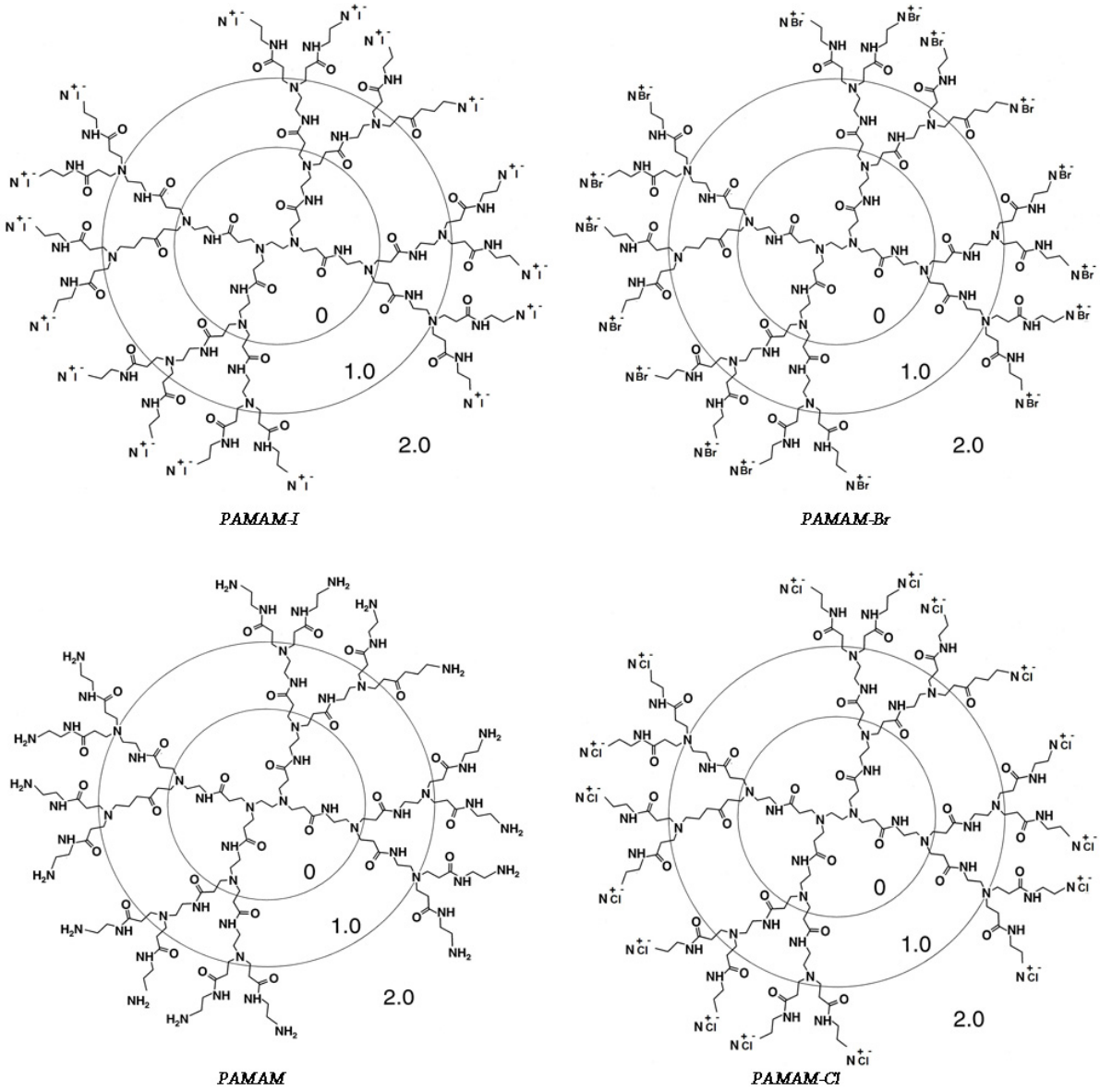

Fig. 1. Chemical structure of PAMAM and modified PAMAM.

2.5. Antimicrobial tests

All bacteria (*Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*) were incubated in nutrient broth culture under aerobic conditions at 37°C for 24 h according to Ahmadi Jebelli et al. [12]. The bacterial inoculums were adjusted to match the 0.5 McFarland turbidity standards. In order to determine the antimicrobial activity of the dendrimer, different concentrations of them (15, 30, 45, and 60 μM) were loaded on the sterile blank paper disks (6 mm diameter) and placed in the center of Mueller–Hinton agar inoculated with test organisms at 37°C for 24 h, which is described in reference [12]. The disk diffusion tests were conducted in triplicates, and the results were presented in terms of the mean average. The MIC and MBC of dendrimers against the target culture bacteria were determined in a manner according to CLSI guidelines [20,21].

3. Results and discussion

3.1. Characterization of PAMAM and modified PAMAM

3.1.1. NMR test

The C-13 NMR spectrum of the PAMAM dendrimer displays a carbonyl resonance at 176 ppm and $–CH_2$ resonances from 29 to 58 ppm, whereas the C-13 NMR spectrum of quaternary ammonium salts of the PAMAM dendrimer shows an additional carbonyl resonance at 155–163 ppm, which is attributed to the urea carbonyl of the quaternary ammonium salts of the PAMAM dendrimer (Fig. 2). Besides, appearing new resonances from 13 to 34 ppm in the C-13 NMR spectrum of the modified dendrimer suggests that long alkyl chains and a CH_3 have been added to the dendrimer [22]. Degree of branching (DB) of dendrimers were calculated according to the equation used by Kanai et al. [10]. The calculated DB

for all dendrimers are between 42% and 47%, which indicate dendrimers natures and are in the range of HB polymer. Similar results have been stated by Kanai et al. [10].

3.1.2. FTIR test

Fig. 2 shows the FTIR spectra of the pure PAMAM and modified PAMAM dendrimer in the region from 1,000 to 4,000 cm^{-1}. The PAMAM dendrimer shows an N–H band at 3,276 cm^{-1}, a C=O band at 1,642 cm^{-1}, and an N–H bending at 1,543 cm^{-1}. In the FTIR spectrum of the modified PAMAM, these bands were shifted to 3,418, 1,654, and 1,551 cm^{-1}, respectively. The PAMAM dendrimer exhibits weak bands around 2,800–3,000 cm^{-1} whereas the modified PAMAM showed strong bands around 2,800–3,000 cm^{-1}. FTIR spectra indicated that long alkyl groups were added to the PAMAM dendrimer ensuring successful converting PAMAM dendrimer to quaternary ammonium dendrimer salts (Fig. 3) [23].

3.1.3. DLS test

Antimicrobial property is one of the key parameters for microbial uptake by a substance with a small size and large specific surface. In order to characterize the self-assembled structure of the dendrimer, DLS was conducted. The histogram analysis of the DLS (Fig. 4) indicates that the mean diameter of the dendrimers ranges from 4 to 6 nm.

3.2. Antimicrobial properties

Antibacterial activities of dendrimers containing chloride, bromide, and iodide as counter anion and their mono-functional counterpart were investigated on gram-positive and gram-negative bacteria. Table 2 shows the results of measurement of MIC and MBC values of dendrimers against *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*. The results prove that both generations of PAMAM dendrimer pose antimicrobial activity and can be used as an antimicrobial agent. Previous studies have revealed that bacteria destroyed by antimicrobial agents is performed through one of methods of bacterial cell membrane damage, spatial deformation, destruction of bacterial enzymes, damage to chromosomes, and lysis of bacteria cell wall [24]. This property can be attributed to the presence of amine groups at the end of the dendrimer structure in response to the negatively charged membrane or cytoplasm of microorganisms, resulting in bacterial cell wall damage and thus preventing bacterial activity [24,25]. The antimicrobial activity of dendrimers is mainly limited to its effect on bacterial membrane permeability. However, there is still no experimental data to support this proposal. It seems that each dendrimer affects the bacterial cell wall with a different approach. It has been expressed that in the PAMAM dendrimers with amine terminal groups, the amine groups form nanopores in the protective fat layer of bacteria membrane, causing rupture and cell lysis [11,26].

Considering antibacterial assessment, it is clear that PAMAM dendrimer is effective as an antibacterial agent against both gram-positive and gram-negative bacteria. For confirming the antimicrobial effect of the amine groups,

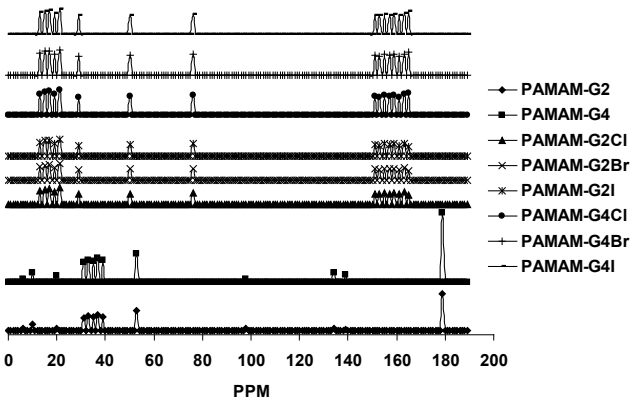

Fig. 2. C-13 NMR spectrum of (a) PAMAM and (b) modified PAMAM dendrimer.

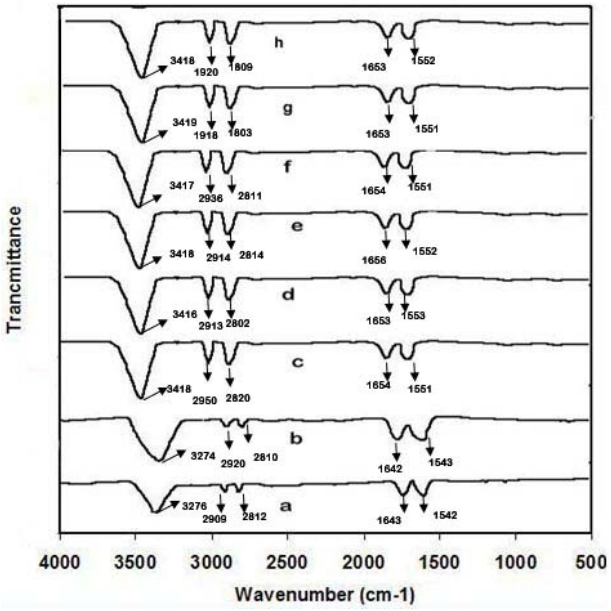

Fig. 3. FTIR spectrum of PAMAM and modified PAMAM dendrimer PAMAM-G_2 (dendrimer order based on Table 1).

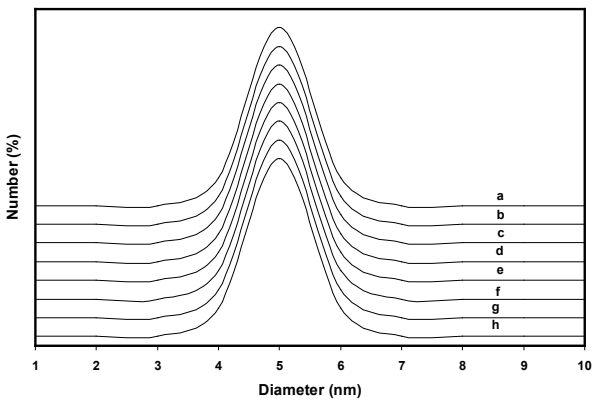

Fig. 4. DLS analysis of PAMAM and modified PAMAM dendrimers (dendrimer order based on Table 1).

Lopez et al. [13] assessed the antimicrobial activity of PAMAM dendrimer on gram-positive and gram-negative bacteria. The results of their study indicate the antimicrobial activity of this dendrimer against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* bacteria. However, it is obvious that gram-positive bacteria are more sensitive against antimicrobial activity of dendrimer compared with gram-negative bacteria. The main difference between gram-positive and gram-negative bacteria is in their cell wall and the amount of the peptidoglycan membrane material. Since the thickness of the peptidoglycan membrane in gram-positive bacteria is more than gram-negative bacteria, it is expected that the former show more resistance to antimicrobial agents [24,27]. However, the results indicate that studied gram-positive bacteria are more susceptible. Mainly the type of dendrimer core, surface charge, and functionality, three-dimensional structure and size of the dendrimer are key factors that affect its antimicrobial activity. PAMAM dendrimers with amine end groups have the antimicrobial activity against both gram-positive and gram-negative bacteria [11,13]. Ortega et al. [28] reported that cationic dendrimers with end amine and ammonium groups have stronger antimicrobial activity against gram-positive bacteria rather than gram-negative bacteria. This difference in antimicrobial activity of dendrimers is because of the special structure of the cell wall of bacteria. Gram-positive bacteria cell wall consists of a thick layer (about 20–50 nm) of peptidoglycan cross-link that limits the interaction between the cell membrane and the functional groups and impairs the dendrimer penetration. It is expected, therefore, to show more resistance to antimicrobial agents. Nevertheless, the cell wall of gram-negative bacteria consists of a thin layer (10 nm) of peptidoglycan and is expected to show less resistance to antimicrobial agents. However, this type of bacteria has an additional outer membrane, which makes the bacteria resistant against many external factors. This structure possesses a unique component called lipopolysaccharide, which increases negative charge of the cell membranes and is essential for the structural integrity and survival of bacteria. Hence, PAMAM dendrimer molecule is not probably able to effectively interact with this layer and cause its instability and degradation [29]. In fact, the outer membrane of gram-negative bacteria acts as a strong barrier against foreign substances [26]. Neu [30] declared that the antimicrobial activity of dendrimers is because of their ability to increase bacterial membrane permeability, which ultimately higher concentrations of dendrimers lead to complete degradation of the bacterial membrane and its death. In addition, binding dendrimer nanostructure and bacterial cell through electrostatic bonds occur because of the surface negative charge of bacterium and positive charge of dendrimer.

Another important point in the antimicrobial activity of dendrimers is the number of functional groups in its structure. Any increase in the generation may cause an increase in the number of quaternary groups, and thus, the system should be more potent. Therefore, it is expected that the dendrimer G4 (64 terminal groups) has a greater antimicrobial activity against microorganisms compared with dendrimer G2 (16 terminal groups) because of the prevalence of terminal amine groups. But, the results (Tables 2 and 3) do not impede considerable and dramatic differences in the diameter of inhibition zone, MIC, or MBC of dendrimers. In fact, it is concluded that although the terminal functional groups play an important role in the antimicrobial activity of dendrimers, there are other factors that are important in the interaction of dendrimer with bacterial membrane and the impact of its functional groups and ultimately affect its antimicrobial activity. The dendrimer size is among the important factors affecting the antimicrobial activity of dendrimer, because it is effective on the ability of dendrimer in penetrating the bacteria wall. As is clear by increasing the PAMAM dendrimer from G2 to G4, the number of terminal amine groups has increased from 16 to 64. Therefore, reciprocal enhancing the antimicrobial activity is reasonable. However, as it is obvious, along with increasing the amine groups, the molecular dimensions of dendrimer have also increased. It was found that the size measured by DLS was around 4–6 nm. Therefore, they are small enough to cross the cell wall of both groups of bacteria. Hence, this almost twice increase in the size of molecule has an important role in the dendrimer penetration into the bacterial membrane and impacts its life. In this regard, Lopez et al. [13] reported that PAMAM dendrimer G3 had much greater antimicrobial activity compared with PAMAM dendrimers G5. Similarly, Ortega et al. [28] showed that the antimicrobial activity of the lower dendrimer generations was much more than the higher generations of carbosilane dendrimer.

The counter anion nature could be an important factor in the biocide efficiency of dendrimers. According to the MIC values reported in Table 2, The PAMAM dendrimer and modified PAMAM dendrimer with halogens showed a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria presented in Fig. 5 for *Klebsiella oxytoca*. To examine the antimicrobial effects of all ammonium salts against gram-positive and gram-negative bacteria, the disc diffusion method was used. The compounds showed a zone of inhibition reported in Table 3 and Fig. 5.

Table 2
MIC and MBC test of antimicrobial efficiency of PAMAM and modified PAMAM against *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*

Type of dendrimer	Type of effect	*S. aureus*	*B. subtilis*	*K. oxytoca*	*E. coli*
PAMAM-G_2	MIC	98	99	110	113
	MBC	105	112	127	129
PAMAM-G_4	MIC	87	88	92	96
	MBC	100	103	121	125
PAMAM-G_2Cl	MIC	63	61	68	70
	MBC	74	77	80	86
PAMAM-G_2Br	MIC	61	55	65	68
	MBC	70	74	77	82
PAMAM-G_2I	MIC	55	53	57	58
	MBC	66	68	73	77
PAMAM-G_4Cl	MIC	61	56	65	68
	MBC	71	74	77	83
PAMAM-G_4Br	MIC	58	53	60	64
	MBC	67	70	75	79
PAMAM-G_4I	MIC	52	50	55	57
	MBC	64	67	71	75

Table 3
Disc diffusion results of PAMAM and modified PAMAM dendrimers against *E. coli*, *K. oxytoca*, *B. subtilis*, and *S. aureus*

	Concentration, μM	Zone of inhibition, mm			
		S. aureus	*B. subtilis*	*K. oxytoca*	*E. coli*
PAMAM-G_2	15	11	9	7	6
	30	14	11	9	8
	45	18	19	14	12
	60	20	22	17	16
PAMAM-G_4	15	14	11	9	8
	30	16	12	11	9
	45	19	20	15	14
	60	21	23	18	17
PAMAM-G_2Cl	15	15	12	10	9
	30	17	14	12	11
	45	20	21	16	15
	60	23	24	20	18
PAMAM-G_2Br	15	16	13	11	10
	30	18	14	12	11
	45	20	22	17	15
	60	24	25	21	19
PAMAM-G_2I	15	18	16	13	12
	30	20	17	15	12
	45	23	25	18	17
	60	27	28	23	22
PAMAM-G_4Cl	15	17	15	12	11
	30	19	17	14	12
	45	21	24	18	16
	60	25	27	22	21
PAMAM-G_4Br	15	19	17	14	13
	30	21	19	16	14
	45	22	25	20	17
	60	26	28	23	22
PAMAM-G_4I	15	22	20	16	15
	30	23	23	19	17
	45	25	27	21	19
	60	31	28	26	25

Assessing the inhibition zone reveals that the dendrimers used have antimicrobial activity, and by increasing concentrations of antimicrobials, a larger inhibition zone is developed around the disc. This situation reflects the strength of the antibacterial substance at higher concentrations. The comparison between the initial and modified dendrimers indicated that the latter one has a greater diameter growth. The dendrimer containing iodide as the counter anion is approximately more effective against both gram-positive and gram-negative bacteria than dendrimer with chloride as the counter anion. Since iodides, compared with chlorides, form weaker anionic pairs with ammonium units, there would be more exposed cations and then stronger electrostatic attraction to the negatively charged bacterial membranes [31]. However, because of the different nature of the ammonium units presented on dendrimer, it seems to be more hydrophilic than the corresponding counterpart, which decreases its biopermeability and thus the antibacterial activity [32]. Therefore, the most inhibition of bacteria was obtained with G_2I and G_4I.

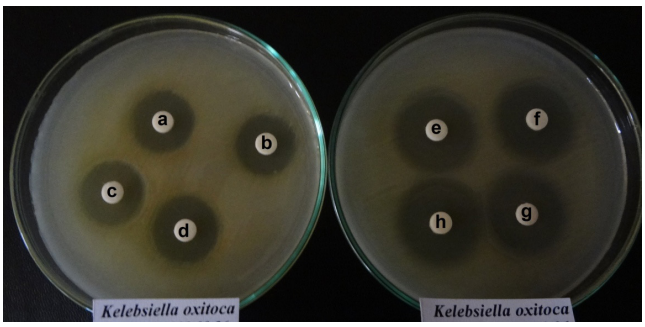

Fig. 5. Growth inhibition of *Klebsiella oxytoca* by PAMAM and modified PAMAM dendrimers: (a) PAMAM-G_2, (b) PAMAM-G_4, (c) PAMAM-G_2Cl, (d) PAMAM-G_2Br, (e) PAMAM-G_2I, (f) PAMAM-G_4Cl, (g) PAMAM-G_4Br, and (h) PAMAM-G_4I.

3.3. Antimicrobial efficacy and contact time

The PAMAM and modified PAMAM prepared as described above were tested for antimicrobial efficacy against *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*. 0.1 g of dendrimer was added to 100 mL of bacterial suspension containing 10^7–10^8 CFU mL^{-1} buffered at pH = 7 in a 250-mL conical flask. After concussion with 200 rpm at 37°C for 1, 5, and 10 min, 0.5 mL of the various bacterial suspensions were placed in sterile test tubes, each containing 4.0 mL of sterile phosphate buffer and 0.5 mL of sterile 0.1 N sodium thiosulfate to quench any probably presented oxidative free chlorine, and vortexed for several seconds. The mixed bacterial suspensions were serially diluted, and 100 mL of each dilution was placed onto a nutrient agar plate. Bacterial colonies on the agar plates were counted after incubation at 37°C for 24 h. Bacterial reduction is reported according to the following equation [3]:

$$\text{Log reduction of bacteria} = \log N_1/N_2 \quad (1)$$

where N_1 is the number of bacteria counted from the original bacterial suspension, and N_2 is the number of bacteria counted from each sample.

The antimicrobial efficacies of dendrimers were tested by challenging with gram-positive and gram-negative bacteria were summarized in Table 4. As can be seen from Table 4, contact time is not effective parameter for antimicrobial test of PAMAM and modified PAMAM against the studied bacteria.

4. Conclusion

PAMAM (G_2 and G_4) dendrimers were modified into quaternary ammonium salts using halogen groups (Cl, Br, I) and characterized with FTIR, C-13 NMR, and DLS analysis. Water samples were collected from rural drinking water sources.

Table 4
Effect of contact time on antimicrobial efficacies of PAMAM and modified PAMAM against *E. coli*, *K. oxytoca*, *B. subtilis*, and *S. aureus*

	Contact time, min	Log reduction of *S. aureus*	Log reduction of *B. subtilis*	Log reduction of *K. oxytoca*	Log reduction of *E. coli*
PAMAM-G_2	1	3.3	3.0	2.5	2.4
	5	3.3	3.0	2.5	2.4
	10	3.3	3.0	2.5	2.4
PAMAM-G_4	1	3.5	3.1	2.7	2.5
	5	3.5	3.1	2.7	2.5
	10	3.5	3.1	2.7	2.5
PAMAM-G_2Cl	1	3.9	3.3	3.0	2.5
	5	3.9	3.3	3.0	2.5
	10	3.9	3.3	3.0	2.5
PAMAM-G_2Br	1	4.0	3.5	3.0	2.7
	5	4.0	3.5	3.0	2.7
	10	4.0	3.5	3.0	2.7
PAMAM-G_2I	1	4.4	3.9	3.2	2.9
	5	4.4	3.9	3.2	2.9
	10	4.4	3.9	3.2	2.9
PAMAM-G_4Cl	1	4.3	3.8	3.2	2.6
	5	4.3	3.8	3.2	2.6
	10	4.3	3.8	3.2	2.6
PAMAM-G_4Br	1	4.5	3.9	3.6	2.9
	5	4.5	3.9	3.6	2.9
	10	4.5	3.9	3.6	2.9
PAMAM-G_4I	1	4.8	4.2	3.7	3.3
	5	4.8	4.2	3.7	3.3
	10	4.8	4.2	3.7	3.3

Using differential biochemical tests, bacteria were isolated and identified. The bacteria isolated from water sources were *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, and *Staphylococcus aureus*. The antimicrobial activity of PAMAM dendrimer (G_2 and G_4) and modified PAMAM dendrimers (G_2Cl, G_2Br, G_2I, G_4Cl, G_4Br, and G_4I) against gram-positive and gram-negative bacteria was examined by calculating MIC, MBC, and the disc diffusion method. We found that quaternary ammonium salts exhibit more antimicrobial efficacy against bacteria. The greatest antibacterial effects were obtained by G_4I and can be considered for further studies in the field of water disinfection and used as an antibacterial alternative in this regard.

Acknowledgments

The authors greatly acknowledge Kurdistan University of Medical Sciences for financial supports, laboratory, and material preparation. The authors wish to thank Dr. Mohammad Sadeghi for performing laboratory antimicrobial tests.

References

[1] Q. Li, S. Mahendra, D.Y. Lyon, L. Brunet, M.V. Liga, D. Li, Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications, Water Res., 42 (2008) 4591–4602.

[2] P. Dallas, V.K. Sharma, R. Zboril, Silver polymeric nanocomposites as advanced antimicrobial agents: classification, synthetic paths, applications and perspectives, Adv. Colloid Interface Sci., 166 (2011) 119–135.

[3] M. Lackner, J.-P. Guggenbichler, Composite Material with a Supporting Material and an Antimicrobial Agent, US Patent 20, Vol. 150, 2015, pp. 104–488.

[4] M. Mangale Sapana, G. Chonde Sonal, P. Raut, Use of *Moringa oleifera* (drumstick) seed as natural absorbent and an antimicrobial agent for ground water treatment, Res. J. Recent Sci., 1 (2012) 31–40.

[5] A. Maleki, M. Ahmadi Jebelli, B. Hayati, H. Daraei, F. Gharibi, Antimicrobial effect of poly (amidoamine)-G2 and G4 dendrimers on some bacteria in water resources, J. Mazandaran Uni. Med. Sci., 25 (2015) 56–68.

[6] H. Tan, R. Ma, C. Lin, Z. Liu, T. Tang, Quaternized chitosan as an antimicrobial agent: antimicrobial activity, mechanism of action and biomedical applications in orthopedics, Int. J. Mol. Sci., 14 (2013) 1854–1869.

[7] A.T. Doolittle, Synthesis and Characterization of Silver Nanoparticle Impregnated Pourous Materials for Antimicrobial Water Filtration and Surface Enhanced Raman Scattering, MS Dissertation, University of Maine, Orono, ME 04469, United States, 2013.

[8] M. Sadeghi-Kiakhani, M. Arami, K. Gharanjig, Application of a biopolymer chitosan-poly (propylene) imine dendrimer hybrid as an antimicrobial agent on the wool fabrics, Iran. Polym. J., 22 (2013) 931–940.

[9] S.S. Abkenar, R.M.A. Malek, Preparation, characterization and antimicrobial property of cotton cellulose fabric grafted with poly (propylene imine) dendrimer, Cellulose, 19 (2012) 1701–1714.

[10] T. Kanai, D. Thirumoolan, R. Mohanram, K. Vetrivel, K. Anvar Basha, Antimicrobial activity of hyperbranched polymers: synthesis, characterization, and activity assay study, J. Bioact. Compat. Polym., 30 (2015) 145–156.
[11] M.K. Calabretta, A. Kumar, A.M. McDermott, C. Cai, Antibacterial activities of poly(amidoamine) dendrimers terminated with amino and poly(ethylene glycol) groups, Biomacromolecules, 8 (2007) 1807–1811.
[12] M. Ahmadi Jebelli, E. Kalantar, A. Maleki, H. Izanloo, F. Gharibi, H. Daraei, B. Hayati, E. Ghasemi, A. Azari, Antimicrobial activities of the polypropylene imine dendrimer against bacteria isolated from rural water resources, Jundishapur J. Nat. Pharm. Prod., 10 (2015) e20621.
[13] A.I. Lopez, R.Y. Reins, A.M. McDermott, B.W. Trautner, C. Cai, Antibacterial activity and cytotoxicity of PEGylated poly(amidoamine) dendrimers, Mol. Biosyst., 5 (2009) 1148–1156.
[14] C.Z. Chen, N.C. Beck-Tan, P. Dhurjati, T.K. VanDyk, R.A. Larossa, S.L. Cooper, Quaternary ammonium functionalized poly(propylene imine) dendrimers as effective antimicrobials: structure–activity studies, Biomacromolecules, 1 (2000) 473–480.
[15] S. Charles, N. Vasanthan, D. Kwon, G. Sekosan, S. Ghosh, Surface modification of poly(amidoamine) (PAMAM) dendrimer as antimicrobial agents, Tetrahedron Lett., 53 (2012) 6670–6675.
[16] E. Murugan, I. Pakrudheen, New amphiphilic poly(quaternary ammonium) dendrimer catalyst for effective reduction of citronellal, Appl. Catal., A, 439–440 (2012) 142–148.
[17] Y. Xue, H. Xiao, Y. Zhang, Antimicrobial polymeric materials with quaternary ammonium and phosphonium salts, Int. J. Mol. Sci., 16 (2015) 3626–3655.
[18] J.A. Whiteford, Quaternary Ammonium Compounds, Conjugated to Polymers, Used as Microbiocides, Google Patents, 2012.
[19] M.A. Wikler, Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement, Clinical and Laboratory Standards Institute, 2010.
[20] A.A. Aamer, M.M. Abdul-Hafeez, S.M. Sayed, Minimum inhibitory and bactericidal concentrations (MIC and MBC) of honey and bee propolis against multi-drug resistant (MDR) *Staphylococcus sp*. isolated from bovine clinical mastitis, Altern. Integr. Med., 4 (2014) 1–5.
[21] W. Fabry, H.-J. Kock, In-vitro activity of polyhexanide alone and in combination with antibiotics against *Staphylococcus aureus*, J. Hosp. Infect., 86 (2014) 68–72.
[22] J. Hu, T. Xu, Y. Cheng, NMR insights into dendrimer-based host–guest systems, Chem. Rev., 112 (2012) 3856–3891.
[23] Z. Gu, M. Wang, Q. Fang, H. Zheng, F. Wu, D. Lin, Y. Xu, Y. Jin, Preparation and in vitro characterization of pluronic-attached polyamidoamine dendrimers for drug delivery, Drug Dev. Ind. Pharm., 41 (2015) 812–818.
[24] S. Salimpour Abkenar, R. Malek, S. Taher, Effect of poly (propylene imine) dendrimer nano structure on antimicrobial property of cotton fabric, Res. Med., 36 (2012) 11–18.
[25] X.F. Wang, S.L. Zhang, L.Y. Zhu, S.Y. Xie, Z. Dong, Y. Wang, Enhancement of antibacterial activity of tilmicosin against *Staphylococcus aureus* by solid lipid nanoparticles in vitro and in vivo, Vet. J., 191 (2012) 115–120.
[26] B. Wang, R.S. Navath, A.R. Menjoge, B. Balakrishnan, R. Bellair, H. Dai, R. Romero, S. Kannan, R.M. Kannan, Inhibition of bacterial growth and intramniotic infection in a guinea pig model of chorioamnionitis using PAMAM dendrimers, Int. J. Pharm., 395 (2010) 298–308.
[27] P.R. Murray, K.S. Rosenthal, M.A. Pfaller, Medical Microbiology. 6th ed., Mosby Elsevier, New York, 2009.
[28] P. Ortega, J.L. Copa-Patiño, M.A. Munoz-Fernandez, J. Soliveri, R. Gomez, F.J. de la Mata, Amine and ammonium functionalization of chloromethylsilane-ended dendrimers. Antimicrobial activity studies, Org. Biomol. Chem., 6 (2008) 3264–3269.
[29] K. Winnicka, M. Wroblewska, P. Wieczorek, P. Tomasz Sacha, E.A. Tryniszewska, The effect of PAMAM dendrimers on the antibacterial activity of antibiotics with different water solubility, Molecules, 18 (2013) 8607–8617.
[30] H.C. Neu, The crisis in antibiotic resistance, Science, 257 (1992) 1064–1073.
[31] K. Midura-Nowaczek, A. Markowska, Antimicrobial peptides and their analogs: searching for new potential therapeutics, Perspect. Medicin. Chem., 6 (2014) 73–80.
[32] D. Staneva, E. Vasileva-Tonkova, M.S.I. Makki, T.R. Sobahi, R.M. Abdel-Rahman, A.M. Asiri, I. Grabchev, Synthesis, photophysical and antimicrobial activity of new water soluble ammonium quaternary benzanthrone in solution and in polylactide film, J. Photochem. Photobiol., B, 143 (2015) 44–51.