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The antibacterial effect of G3-poly-amidoamine dendrimer on gram negative and gram positive bacteria in aqueous solutions

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ABSTRACT

Dendrimers are symmetric, round and ramose macromolecules made up of monomers which have a certain structural order. The aim of this study was to evaluate the antibacterial effects of different concentrations of G3-poly-amidoamine dendrimer (G3-PAD) on 7 species of gram-negative and gram-positive bacteria in aqueous solutions. Different concentrations of G3-PAD were prepared and from each bacterial species, a suspension with a different pH was prepared according to the McFarland 0.5 standard. Bacteria were left in a 37°C incubator and shaker from 0 to 60 min, and were cultivated on a Mueller-Hinton Agar plate. The bacteria that grew on each plate were counted and analyzed. The results showed that the antibacterial effect of G3-PAD in aqueous solutions was directly related to dendrimer concentration, contact time and pH. The strongest effect on Salmonella and Bacillus Subtilis was at pH = 6.5 and 7.5 and contact time 45 min, and in pH = 9, contact time 30 min; for Enterococcus faecalis in all three pH, it was 60 min; for Staphylococcus Aureus in pH = 6.5 and 7.5, 60 min and in pH = 9, 30 min; for E. coli in pH = 6.5 and 7.5, 60 min and in pH = 9, 45 min. G3-PAD showed its strongest effect on Shigella bacteria in pH = 6.5, contact time 60 min. In pH = 7.5 there was no optimum condition and in pH = 9, the optimum contact time was 60 min. The strongest effect of G3-PAD on all species was seen in 2000 ppm concentration. These results show a new strategy to improve bacteria elimination from aqueous solutions. However, safely using dendrimers for disinfecting drinking water still needs more research.

Keywords: G3-poly-amidoamine dendrimer; Gram-positive bacteria; Gram negative bacteria; Polymer; Aqueous solutions

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1. Introduction

Nowadays, most world countries are facing difficulty in supplying drinking water for their populations [1–6]. According to the report of the world meters site in 2016, close to 645 million people in the world, did not have access to safe drinking water [7]. World Health Organization (WHO) reported that drinking water should be free of thermal coliform and total coliform in 100 ml samples [8]. Water disinfection has caused the increase of global mortality and control dangerous water-borne diseases such as cholera, typhoid, diarrhea, dysentery, poliomyelitis, tuberculosis, Pontiac fever etc. [9–11]. These facts showed the necessity of disinfecting drinking water [12,13]. Up to now, different chemical and physical methods have been used for water sanitation. The most common methods for water sanitation are chlorine and its compounds. Some research has shown that using chlorine might be ineffective on some microorganisms and viruses [14,15]. Chloramines produce very small amounts of trihalomethane and the cost of using chlorine dioxide is higher than chlorine [16]. Therefore using these chemical disinfectants because of their low efficiency, high cost and producing dangerous by products has decreased. Nano particles can have an important role in treating water. Four classes of nano-chemicals are used for treating water. These include dendrimers, metal nano particles and carbon nano tubes. Dendrimers have a wide range of physical and chemical characteristics which makes them valuable for water treatment [17,18]. Dendrimers have different generations, different dimensions and molecular masses, which is controlled in the synthesis process [19]. The branches have an organized and uniform structure, which is very effective on the characteristics of the dendrimers. One of the most important units in determining the characteristics and applications of a dendrimer are the chemical groups bound to the branching units. Superficial groups binding to the dendrimer molecules are very diverse. These groups include amine, carboxylate, hydroxyl, methyl ester and the hydrophobic C6 branch. Two groups of the mostly used dendrimers are polyamidoamine (PAD) and polypropylenimine (PPI), which have been made available commercially [20,21]. Dendrimers have been used for different purposes due to their unique structure; including pharmaceutics, transfer of medicinal compounds, identifying cancerous cells, chemotherapy, antimicrobial compounds and pollutant absorbers in aqueous solutions. These particles are able to fit various molecules and a wide range of water-soluble including cations, anions and organic compounds among their branches and, encapsulate them [17,22].

Dendrimers have disadvantages as well, which are toxic in higher generations. The toxicity of particles depends on chemical composition. There is a little known about their possible adverse effects on both humans and the environment. To the best of our knowledge, there have been a few studies investigating the mammalian toxicity of dendrimers, therefore is some conflicting evidence regarding their biological. The developmental neurotoxicity of poly-amidoamine dendrimer exposure has not been adequately studied. Dendrimers elicited many different gene expression changes in human cells, including epidermal growth factor receptor. Also may affect the mitochondrial activity, apoptosis, and neuronal differentiation. High volume dendrimers cannot penetrate the cell membranes and reach the effective sites for pre-determined antimicrobial activity. Therefore, the antimicrobial characteristics of the 3rd generation polyamidoamine dendrimers (PAD-G3) are much stronger than 5th generation polyamidoamine dendrimers [23]. Research shows that dendrimers can have more efficiency than other antimicrobial agents. The antimicrobial effects of dendrimers are attributed to the electrostatic interactions between the cationic parts of the dendrimers and the anionic parts of the surface of bacterial cells which is associated with the disturbance in the cell membrane and eventually cellular lysis [24]. When dendrimers enter bacterial solutions, it replaces the bacterial superficial binomial ions, such as calcium and magnesium. Then it attaches the phospholipid membranes with the negative charge and causes slight changes in the permeability of the membrane. The higher concentrations of the dendrimer causes denaturation of membrane proteins and cause perforation in phospholipids. In this stage the high permeability of the membrane cause potassium ion leakage. This concentration of dendrimers has an inhibitory effect. If the concentration of dendrimer increases further, it can unstabilize the membrane structures [25]. The antimicrobial characteristics of dendrimers are related to key factors such as the type of dendrimer nucleus, its surface charge and functional groups, the three-dimensional structure of the dendrimer and its size. Dendrimers have low toxicity to eukaryotic cells, because of their similarity to body proteins [23].

The aim of this study was to evaluate the possibility of using the G3-PAD as a disinfectant and evaluating its effects on gram negative bacteria such as Escherichia coli, Klebsiella Oxytoca, Shigella dysentery and Salmonella; and gram positive bacteria such as Bacillus subtilis, Enterococcus faecalis and Staphylococcus aureus in aqueous environments.

2. Materials and methods

This was an applied experimental study, in which the antibacterial effects of G3-PAD on gram negative and gram positive bacteria in aqueous solutions were evaluated at laboratory scale.

2.1. Preparing the antibacterial compound and its characteristics

The antibacterial compound used in this study was G3-PAD, which was purchased from the Amir Kabir Industrial University, Iran. The concentration of the original nano-dendrimer (G3-PAD) was 2% and the 0.0001, 0.001, 0.01 and 0.1 concentrations were prepared by the serial method and by double distilled water as the solvent. G3-PAD has a molecular formula of $C_{302}H_{608}N_{122}O_6$, a molecular mass equal to 6906 and a diameter equal to 3.6 nm and includes 32 surface groups. The chemical structure of G3-PAD is shown in Fig. 1.

2.2. Preparation of bacterial species

The bacteria under investigation in this research were gram negative bacteria including Escherichia coli, Kleb-

siella Oxytoca, Shigella dysentery and Salmonella; and gram positive bacteria such as Bacillus subtilis, Enterococcus faecalis and Staphylococcus aureus. The standard species of these bacteria were purchased from the Iran Industrial Research Center. All of these bacteria were heated to 37°C for 24 h prior to use, under aerobic conditions and in an agar nitrite environment. The water used was sterilized by autoclave in 121°C for 15 min. A dilution of 10^3 CFU/ml was prepared for each bacterial species. In order to prepare 10^3 CFU/ml bacterial dilutions, bacterial suspensions were prepared from the McFarland 0.5 standard (turbidity equal to 1.5×10^8 bacteria per ml), and from Eq. (1), dilutions of 10^3 CFU / ml, for the required ratios were obtained

$$C_1V_1 = C_2V_2 \quad (1)$$

In this equation, C_1 is the bacterial concentration equal to McFarland 0.5, V_1 is the volume needed for preparing a bacterial concentration equal to 10^3 CFU/ml, C_2 is the bacterial concentration equal to 10^3 CFU/ml, and V_2 is the volume equal to 40 ml (4 dendrimer concentrations, in which each concentrations was transferred to 10 ml water, with a bacterial concentration of 10^3 CFU/ml). 0.27 µL was taken from the equivalent 0.5 McFarland standard and by sterile distilled water its volume was increased to 40 ml and a bacterial dilution of 10^3 CFU/ml was achieved. The procedure was repeated for each bacteria separately.

2.3. Preparing a bacterial dilution according to the McFarland 0.5 standard

In microbiology the McFarland standard is a benchmark for comparing turbidity from bacterial suspensions with the number of bacteria in a specific range. The main McFarland standards are made by mixing a particular amount of barium chloride with sulfuric acid. Mixing these two chemicals produces sediments of barium sulphate which causes turbidity in the solution. The McFarland 0.5 standard is prepared by mixing 0.05 ml of hydrated 1.175% barium chloride ($BaCl_2 2H_2O$) with 9.95 ml 1% sulfuric acid [26].

The number of inoculated bacteria is one of the most important variables which affect the results of this research; thus, the concentration of the inoculated bacterial suspension should be standard. Therefore, for preparing the microbial suspension a few colonies were transferred from a fresh and young bacterial culture to a tube containing sterile salt solution (0.9% salt in distilled water), by a sterile swap. Then the turbidity of the prepared microbial suspension was compared to a McFarland 0.5 standard (turbidity equal to 1.5×10^8 bacteria in each ml [27,28].

2.4. Experiments

To each experimental tube, a bacterial suspension with a specific pH, and 0.5 cc of a specific dilution of dendrimers in completely sterile conditions, was added; in laboratory temperature (23–25°C); and one tube was considered as control. After mixing the dendrimers solution with bacterial

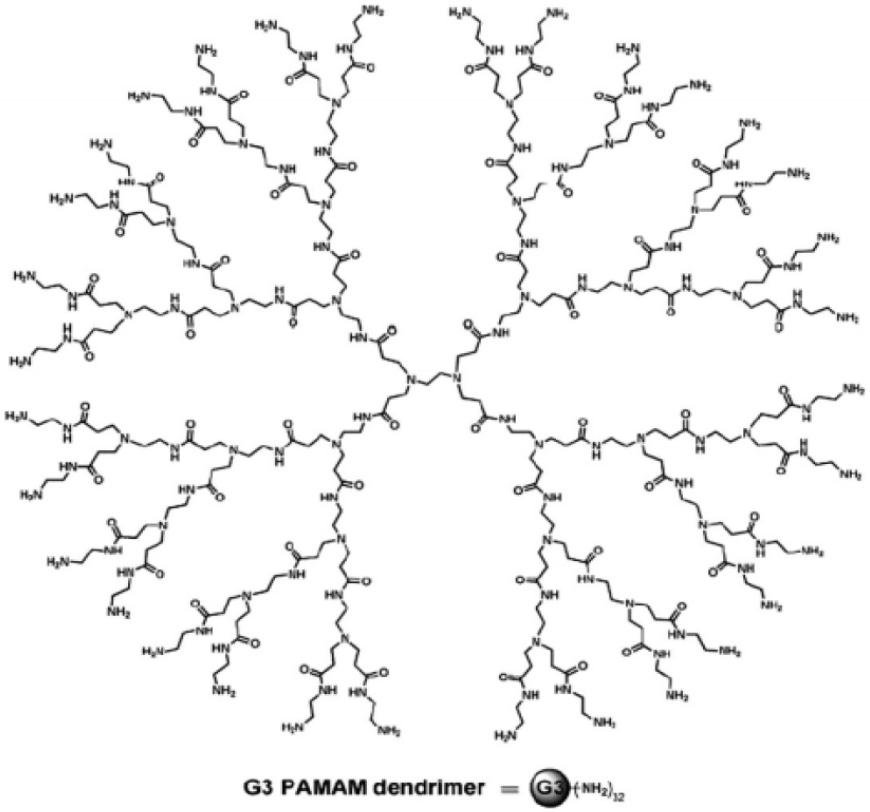

Fig. 1. The chemical structure of G3-PAD.

suspensions and shaking the tubes, in different times which were 0, 15, 30, 45 and 60 min, samples were taken by using a 100 Land a Micro Piper, and each bacteria was cultured linearly on a specific culture medium.

After linear culture of bacteria in the nutrient agar environment, bacteria were kept for 24–48 h in an incubator with 37°C temperature. All of the culture media used in this study were made by the German Merck Company. After heating the bacteria for 24–48 h in the incubator, samples were taken out and the number of colonies that grew on the nutrient Agar medium was counted by a colony counter.

The total number of samples considering the dendrimer concentration factors, stay time, the number of bacteria under study, pH, three repetitions and the control samples were 1323. All experiments we're done according to the guidelines of the Clinical Laboratory and Standards Institute (CLSI) 27].

2.5. Doing the anti biogram test

For determining the diameter for the non-growth circle, the disk diffusion method was used. Blank standard sterile and dry discs with a diameter of 6 mm were impregnated with 50 µg/ml of dendrimer G3-PAD at 20000 ppm concentration. Then the disk was placed on the Muller Hinton Agar culture medium by a sterile plier and was heated in 37°C for 24 h. After this time, the sensitivity or tolerance of bacteria in the mentioned concentration of the dendrimers was determined by measuring the diameter of the circle of non-growth.

3. Results and discussion

The sensitivity of the bacteria to the 2% concentration of the G3-PAD is shown in Table 1. The highest sensitivity was seen in Salmonella bacteria which had the biggest non-growth diameter and the least sensitivity was seen in Klebsiella Oxytoca which had the smallest non-growth diameter.

After several times doing the anti biogram test for the Klebsiella Oxytoca bacteria and not observing a non-growth circle, we concluded that the G3-PAD is not effective on this bacteria and this bacteria is the most tolerant. It seems like the reason for the ineffectiveness of the dendrimer on these bacteria is the structure of the cellular membrane, which is made from a polysaccharide capsule and acts as a strong barrier against the entrance of dendrimers inside the bacteria. Therefore, no further experiments were done on this bacteria. Also, Fig. 2 shows the anti biogram test on the bacteria under investigation for determining their sensitivity to the G3-PAD in Muller Hinton media. In a study done by Maleki et al. about evaluating the antibacterial effects of second and fourth generation PAMAM dendrimers on some bacteria present in water resources in 2015, results showed that PAMAM dendrimers have a similar antibacterial activity against all bacteria and the non-growth diameter for different concentrations of PAMAM-G2 and G4 dendrimers (0.5–500 µg/dl) is respectively 18, 0, 0, 0 mm and 17, 0, 0, 0 for Escherichia coli; 0, 0, 0, 0 mm in both dendrimers for Pseudomonas; 21, 10, 0, 0 mm and 21, 12, 0, 0 mm for Klebsiella; and 34, 22, 18, 10 mm and 35, 22, 18, 11

Table 1
The results of the anti biogram test for determining the sensitivity of bacteria to G3-PAD in the Muller Hinton agar medium

Bacteria	Diameter of non-growth circle (mm)
Escherichia coli	25
Salmonella	35
Klebsiella oxytoca	0
Bacillus subtilis	30
Staphylococcus aureus	30
Shigella dysentery	20
Enterococcus faecalis	20

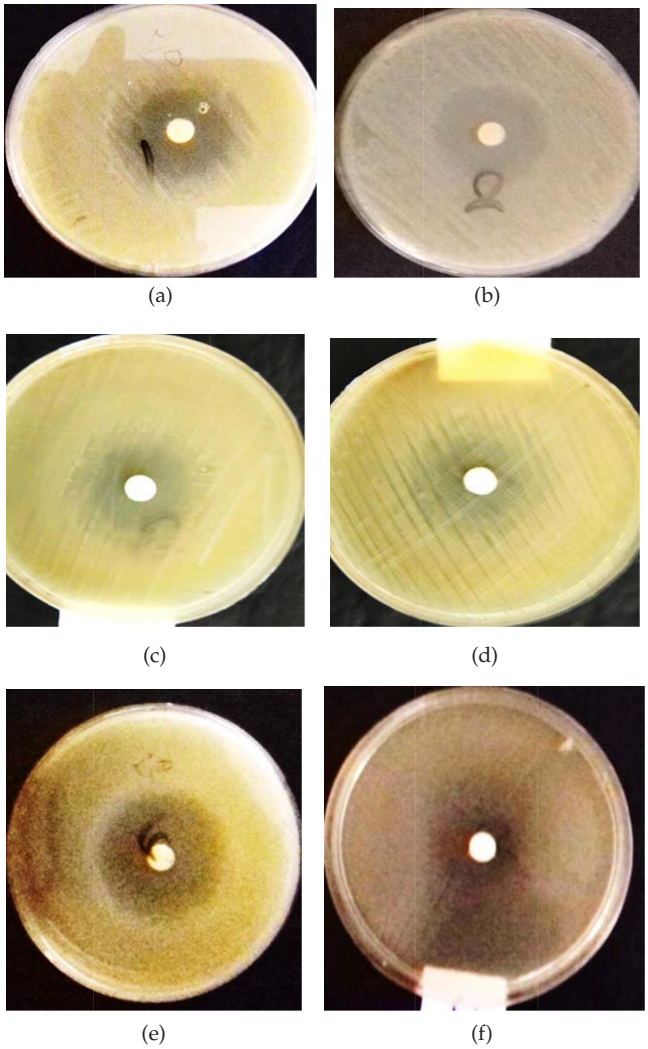

Fig. 2. The antibiogram test on the bacteria under investigation for determining their sensitivity to the G3-PAD in Muller Hinton media. a) Escherichia coli, b) Staphylococcus aureus, c) Bacillus Subtilis, d) Shigella dysentery, e) Salmonella, f) Enterococcus faecalis.

mm for Staphylococcus Aureus; and eventually 24, 19, 17, 7 mm and 25, 21, 17 and 8 mm for Bacillus Subtilis [29]. Also, in study Charles et al. about the antibacterial effect of G3-PAD nano-dendrimers on Escherichia coli and Staphylococcus aureus bacteria results showed that with increased concentrations of nano-dendrimers, the diameter of the non-growth circle in these bacteria, increase as well [30].

The results of counting bacterial colonies on the culture media has been shown in Table 2–7. Table 2 shows the effect of the G3-PAD on Enterococcus Faecalis bacteria in different concentrations, time and pH. The results showed that the best circumstances for the G3-PAD to affect the bacteria is in 3 pHs (6.5, 7.5, 9), 60 min time and 2000 ppm concentration. Also, the greatest effect of dendrimer removal on this bacterium was obtained at pH = 9 due to its growth retardation at high pH. As the concentration of the dendrimer decreases, its effect in preventing the growth of bacteria decreases as well. As the incubator time and shaking of bacteria increases, as a result of more time for the dendrimers to affect the bacteria, bacteria growth decreases and at higher concentrations of the dendrimer, this decrease is very prominent. According to the drinking water microbiology standards (standard 1011) and the absence of Enterococcus faecalis in drinking water, the results were satisfactory.

The effect of the G3-PAD on gram positive Bacillus Subtilis has been shown in Table 3. The results showed that the best circumstances for the G3-PAD to affect this bacteria is at pH = 6.5 and 7.5, in 45 min time; and in pH = 9 in 30 min time and 2000 ppm concentration; and the strongest effect of dendrimer elimination on bacteria was at pH = 9. The results showed that with decrease in dendrimer concentration or in other words by diluting it, the effect of dendrimers on preventing bacterial growth decreases, as well. Also, the results showed with increase in incubator time and shaking, because the dendrimers have more time to affect bacteria, bacterial growth decreases; and in high concentrations this decrease is very prominent. According to microbial standards for drinking water (standard 1011) and the non-existence of Bacillus Subtilis bacteria in drinking water, the results for these bacteria were satisfactory.

Table 4 shows the effect of the G3-PADs on gram positive Staphylococcus aureus bacteria. Results show that the best circumstances for the G3-PAD to affect this bacteria is in pH = 6.5 and 7.5, 60 min time; and in pH = 9, 30 min time and in 2000 ppm concentration. Results showed that with increase in dendrimer concentration, incubator time, shaking and pH; because of the higher concentration, there is more time for dendrimers to affect bacteria; and bacterial growth decreases in

Table 2
The effect of G3-PAD on Enterococcus faecalis bacteria in specific concentrations, times and pHs in aqueous solutions

The count of bacterial growth in different incubator times and shakers $10^8 \times$ CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	890	900	805	570	705	620	260	409	315	95	160	150	0	0	0
200	900	940	880	630	830	730	475	690	570	315	510	306	105	315	170
20	950	970	900	800	900	800	780	805	680	700	770	600	680	700	500
2	1000	990	940	950	950	910	900	910	860	860	900	820	850	880	800
Control sample, pH = 6.5	1000														
Control sample, pH = 7.5	1000														
Control sample, pH = 9	950														

Table 3
The effect of G3-PAD on Bacillus Subtilis in different concentrations, times and pH, in aqueous solutions

The count of bacterial growth in different incubator times and shakers $10^8 \times$ CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	840	890	810	311	270	175	79	55	0	0	0	0	0	0	0
200	890	905	850	517	660	250	312	430	120	200	180	70	50	50	0
20	950	920	890	640	760	750	610	680	600	550	600	525	500	520	450
2	980	940	910	900	890	850	880	850	820	700	800	750	650	800	700
Control sample, pH = 6.5	1000														
Control sample, pH = 7.5	950														
Control sample, pH = 9	900														

Table 4
The effect of the G3-PADs on Staphylococcus aureus bacteria in different concentrations, time and pH in aqueous solutions

The count of bacterial growth in different incubator times and shakers 10^8 × CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	860	900	800	515	610	150	320	255	0	115	65	0	0	0	0
200	900	915	860	735	680	330	475	390	35	220	186	0	45	95	0
20	935	940	890	820	810	675	693	680	515	450	360	320	245	193	200
2	965	960	940	950	890	845	895	850	790	770	800	730	630	750	670
Control sample, pH = 6.5	980														
Control sample, pH = 7.5	1000														
Control sample, pH = 9	950														

Table 5
The effects of G3-PADs on Escherichia coli bacteria in different concentrations, times and pH in aqueous solutions

The count of bacterial growth in different incubator times and shakers 10^8 × CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	900	0	750	350	670	220	100	160	100	20	85	0	0	0	0
200	950	150	790	540	780	380	320	490	210	150	175	50	150	65	0
20	1000	550	830	820	820	720	680	780	670	590	650	500	550	620	250
2	1000	950	850	1000	900	800	1000	870	730	980	800	600	950	780	400
Control sample, pH = 6.5	1000														
Control sample, pH = 7.5	1000														
Control sample, pH = 9	900														

Table 6
The effects of G3-PADs on Shigella bacteria in different concentrations, times and pH in aqueous solutions

The count of bacterial growth in different incubator times and shakers 10^8 × CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	920	940	900	700	810	705	405	520	407	105	220	85	0	50	0
200	930	950	930	780	850	815	520	600	620	317	310	405	140	130	220
20	940	970	940	850	910	900	645	860	830	510	750	705	365	620	610
2	950	980	945	940	950	935	900	900	900	850	870	850	820	850	820
Control sample, pH = 6.5	1000														
Control sample, pH = 7.5	1000														
Control sample, pH = 9	950														

high pH; and therefore, its effect in preventing bacterial growth increases as well. This decreased growth is in accordance with the drinking water microbiology standards, standard 1011, in which the maximum number of staphylococcus aureus bacteria in drinking water should be less than 50 per 100 ml.

The effect of dendrimer G3-PAD on gram negative Escherichia coli bacteria has been shown in Table 5. The best circumstances for the G3-PAD to effect this bacteria was seen in pH = 6.5 and 7.5, in 60 min time; and in pH = 9 in 45 min time and 2000 ppm concentration. Also, the results

Table 7
The effects of G3-PADs on Salmonella bacteria in different concentrations, times and pH in aqueous solutions

The count of bacterial growth in different incubator times and shakers 10^8 × CFU															
Time (min)	0			15			30			45			60		
pH	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9	6.5	7.5	9
Concentration (ppm)															
2000	850	830	750	310	280	50	50	74	0	0	0	0	0	0	0
200	885	910	800	572	520	220	230	230	70	25	85	0	0	0	0
20	920	940	860	730	870	645	562	630	430	345	470	265	170	160	150
2	970	965	920	880	910	810	755	760	780	640	650	670	590	500	440
Control sample, pH = 6.5	1000														
Control sample, pH = 7.5	1000														
Control sample, pH = 9	950														

showed that's the greatest effect of dendrimer removal on bacteria was due to decreased growth at high pH, at pH = 9. According to the drinking water microbiology standards (standard 1011) and the non-existence of Escherichia coli bacteria in drinking water, satisfying results were achieved, in high dendrimer concentrations, high incubation time, high shaking and high pH.

Table 6 shows the effect of the G3-PAD on gram negative Shigella bacteria in different concentrations, times and pH. According to the drinking water microbiology standards (standard 1011) and the non-existence of Shigella bacteria in drinking water, our results showed that the best situation for the dendrimer to affect this bacteria is at pH = 6.5, 60 min time; and 2000 ppm concentration. In pH = 7.5, because the number of bacteria was not zero in different concentrations and experimental times, we did not achieve optimum conditions; and in pH = 9, the time was 60 min and the concentration was 2000 ppm.

The effect of the G3-PAD on gram negative salmonella bacteria has been shown in table 7. The best circumstances for the G3-PAD to effect this bacteria is at pH = 6.5 and 7.5, 45 min time; and in pH = 9 in 30 min time and 2000 ppm concentrations. These results are in accordance with the drinking water microbiology standards (standard 1011) that quote the non-existence of salmonella bacteria in drinking water. Results showed that with decrease in dendrimers concentrations, incubator time, shaking and pH = 6.5, its effect on preventing bacterial growth will decrease as well.

This research showed that G3-PAD nano-dendrimers have sufficient antibacterial properties and can affect gram negative and gram positive bacteria; and this effect is stronger on gram positive and gram negative bacteria. Also, results showed that this chemical has no antibacterial effect on Klebsiella Oxytoca.

A study about the antibacterial effect of the PAMAM-G4 nano-dendrimer showed that this nano-dendrimer has antibacterial effects against Escherichia coli, Bacillus Subtilis, Staphylococcus Aureus, but does not affect Enterobacter Kelvake [31]. Another study done on the antibacterial effects of the PAMAM-G4 dendrimers, showed that this chemical has no antibacterial effect on Pseudomonas Aeruginosa either [32]. Felczak et al. showed that the polypropylene amine G4 dendrimers had the highest antibacterial effect against gram positive bacteria [23]. The high sensitivity of gram-positive bacteria in comparison to gram-negative bacteria maybe related to the interaction mechanisms between the bacterial membrane and the dendrimers or the difference in the structure of the bacterial cell wall. Gram negative bacteria have an external membrane which acts as a barrier and prevents the penetration of big and hydrophobic molecules. On the other side, because the thickness of the peptide and glycon membrane in gram positive bacteria is more than gram negative bacteria, it is expected that gram positive bacteria have more tolerance to antimicrobial agents. Nevertheless, as the results show gram positive bacteria are more sensitive. Often the type of dendrimer nucleus, electrical charge and groups, three-dimensional structure and dendrimer size is among the key factors that affect antimicrobial activity. Polyaminiamine dendrimers with end-amine groups, have antimicrobial activity against gram negative and gram positive bacteria [33–35].

Ortega et al. reported that cation dendrimers with a mine and ammonium end-groups have a stronger antimicrobial effect on gram positive bacteria in comparison to gram-negative bacteria. This difference in antimicrobial activity among dendrimers is related to the specific structure of the bacterial cell wall [36]. Operating groups get absorbed on the surface of bacterial cells and penetrate through the cellular wall. Then they attach the cytoplasmic membrane and destroy it. At this time, electrolytes like potassium and phosphate ions and nuclear material such as DNA and RNA are released from the cell, and this causes the death of the bacterial cell. Amine groups, causes the formation of nano-holes in the lipid layer that protects the bacterial membrane; and this causes the rupture and death of the cell [37]. Although the terminal operating groups have an important role in the antimicrobial activity of the dendrimer, but there are other factors that are effective on the interaction between the dendrimer and the bacterial membrane, and the effect of the operating groups; and eventually influence the antimicrobial effects. The size of the dendrimer is among the important and effective factors on antimicrobial activity, because it is effective on the ability of the dendrimer in penetrating the bacterial wall. Therefore, increase in the molecular dimensions has an important role in the pen-

etration of the dendrimer into the bacterial membrane and its vitality [31,38,39]. Another similar study done by Ortega et al. in 2008, showed that the antimicrobial activity of lower generations of carboxylene dendrimers is much higher than its new generations [40].

Results show that the antibacterial properties of nano-dendrimers in aqueous solutions has a direct relation with increased concentrations of nano-dendrimers and their contact time; and all concentrations of nano-dendrimers in different times, decrease bacteria. At time 0, the G3-PAD nano-dendrimers had little effect on the bacteria under study, but after 60 min, the G3-PAD nano-dendrimers caused significant decrease in bacteria. The present study showed with increase in contact time up to 60 min, Enterococcus faecalis and Shigella were 100% eliminated in a concentration of 2000 ppm, but Bacillus Subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella were eliminated at concentrations ≥ 200 ppm of the G3-PAD. Increase in bacteria elimination efficiency as the contact time increases, shows that this chemical is stable in aqueous solutions. All concentrations of the dendrimer in different times and pH, cause decrease in bacteria and this decrease is higher in higher concentration of the dendrimers. G3-PADs also have adequate anti-bacterial characteristics in low concentrations, because of their tree like characteristics, organized and multi branch structure, empty spaces between branches, high number of operating a mine end-groups and their macromolecules.

Results show that 3rd generation PAMAM have antimicrobial characteristics and can be used as antimicrobial agents. These results are in line with the results of Shahbazi et al. in 2014 about the antimicrobial activity of the 2nd and 5th generation PAMAM [41], and the study done by Gholami et al. about PAMAM-G7 in eliminating Escherichia coli, Klebsiella Oxytoca, Pseudomonas Aeruginosa, Proteus mirabilis, and Staphylococcus aureus bacteria from aqueous solutions [42]. Also, studies about the antibacterial effects of second generation PAMAM have shown that the concentration of 0.5 µg/ml of these chemicals did not have an effect on Escherichia coli and Proteus mirabilis, but with increased concentration up to 500 µg/ml, bacteria such as Escherichia coli, Proteus mirabilis, Bacillus Subtilis, and Staphylococcus aureus were completely eliminated [43]. In contrast to ozone, UV or ClO_2 which have a low shelf time in aqueous solutions and after a few min leave little residuals, the G3-PAD nano-dendrimers can stay in aqueous solutions for long times. Secondary pollution can happen in drinking water distributing networks at any time, and using this chemical can be considered because of its high shelf time as an antimicrobial agent [44].

The present study showed that Escherichia coli and Bacillus Subtilis have lower tolerance against anti-microbial agents than Salmonella Typhi and Shigella dysentery and are eliminated in lower concentrations. As it can be seen in Tables 2–7, as the concentration of nano-dendrimers and their contact time increase, the antimicrobial effect of nano-dendrimers increase as well; and cause significant decrease in bacteria. Also, with increase in the concentration of G3-PADs, the non-growth circle increases significantly. This study also showed that the G3-PAD in low concentrations can stop the growth of Salmonella, Staphylococcus aureus, and Bacillus Subtilis; but for stopping the growth of Shigella, Escherichia coli, and Enterococcus faecalis higher concentrations of the dendrimer is needed.

4. Conclusions

The results of this study show the antibacterial effect of the G3-PAD on bacteria. The strongest effect was on Salmonella and the weakest effect was on Klebsiella Oxytoca. Results show that if contact time and dendrimer concentrations increase, the antibacterial effects of G3-PAD increase as well. Results also show if the selected pH is further away from the optimum pH, because of the joint effect of dendrimers and pH in bacterial elimination, better results are achieved. Finally, although these dendrimers have antimicrobial effects, but their use for disinfecting drinking water still needs more research, and especially more research has to done about its possible toxic effects.

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