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Bioassay Testing the Toxicity of Nano-Structure Polymer (PAMAM G2) as Coagulant Aid in Water Treatment

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ABSTRACT

Poly (amido amine) generation 2.0 (PAMAM G2) has a tree-like structure having different applications and properties, among them is trapping tiny flocks and improving the procedure of coagulation. In this study, the toxicity effect of PAMAM G2 nanodendrimer as a coagulant aid was surveyed through bioassay testing for the first time. For this purpose, PAMAM G2 as a coagulant aid was applied at different doses (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L⁻¹), accompanied by ferric chloride as coagulant in jar test. Then, the toxicity of water extracted from coagulation process and one sample without PAMAM G2 as testament were assessed. In order to measure the acute toxicity, bioassay experiment with *Daphnia magna* was used. It was cultured in specific medium for mass culture. Then, six young generated juveniles plus one as testament were added to each vessel. The bioassay test was performed for 48 h during which each sample was taken and evaluated at time interval of 2, 4, 8, 12, 24 and 48 h. The results showed that the usage of PAMAM G2 as coagulant aid would not lead into toxicity in water.

Key words: Biological assay, coagulation, nanodendrimer, toxicity test, water purification

INTRODUCTION

The availability of drinkable water for human health and the development and progress of societies is essential (Sadani *et al.*, 2011). The purpose of water treatment is to produce a safe and aesthetically pleasing water. This requires that the water be free of harmful chemicals and microbes, as well as have an acceptable taste and odor (Chevalier and McCann, 2008).

A holistic approach to the risk assessment and risk management of a drinking water supply increases confidence in the safety of the drinking water. This approach entails systematic assessment of risks throughout a drinking-water supply from the catchment and its source water through to the consumer and identification of the ways in which these risks can be managed, including methods to ensure that control measures are working effectively (WHO., 2006). Particles in raw water can influence the disinfection process in a negative way. When shielded by particles the disinfectant may not be as effective in inactivating pathogens. Meaning that particles may decrease the efficiency of disinfection, because of which a higher degree of treatment might be required for effective water treatment. Therefore, by the removal of the particles, the efficiency of disinfection can be as effective as possible (HDR Engineering Inc., 2001).

Dendrimers are a class of thoroughly branched polymers characterized by a high chemical versatility. Dendrimers have unique properties such as uniform size, well-defined molecular weight

and tunable surface functionality and solubility. Moreover, the presence of rather large internal cavities makes them interesting for many biological and medical applications (Suarez *et al.*, 2011; Majoros and Baker Jr., 2008). Dendrimer is generally described as a macromolecule characterized by its highly branched 3D structure that provides a high degree of surface functionality and versatility (Buhleier *et al.*, 1978). Dendrimers possess three distinguished architectural components, namely: (i) An initiator core, (ii) Interior layers (generations) composed of repeating units, radically attached to the interior core and (iii) exterior (terminal functionality) attached to the outermost interior generations (Pushkar *et al.*, 2006; Sakthivel and Florence, 2003).

The synthesis procedure used for dendrimer preparation permit almost entire control over the critical molecular design parameters such as size, shape, surface/interior chemistry, flexibility and topology (Frechet *et al.*, 2002). Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared with traditional linear polymers. In solution, linear chains exist as flexible coils; in contrast, dendrimers form a tightly packed ball. This has a great impact on their rheological properties. Lower generation dendrimers, which are large enough to be spherical but do not form a tightly packed surface, have enormous surface areas in relation to volume. In contrast to linear polymers, the intrinsic viscosity of dendrimer solutions does not increase linearly with mass but shows a maximum at a specific generation and then begins to decline (Dufes *et al.*, 2005).

Polymers have been utilized in coagulation/flocculation processes for water purification for at least four decades (Kawamura, 1976). In comparison with alum, some of the advantages flowing from the use of polymers in water treatment are: Lower coagulant dose requirements, less sludge production, less increase in the ionic load of the treated water and reduced level of aluminum in treated water (Nozaic *et al.*, 2001).

Polymers are especially beneficial in coping with the problems of slow-settling flocs in low-temperature coagulation or in treating soft coloured waters, where they improve settle ability and increase the toughness of flocs (Faust and Aly, 1998). The capacity of a treatment facility may be more than doubled with the formation of larger and stronger flocs, the rate of solid and water phase separation can be significantly increased and the dosage of other chemicals be lowered. In addition, the range of water that can be treated is wider. There are disadvantages of course, with higher costs in particular situations and environmental factors being the main concern. There is a greater sensitivity to incorrect dosage, with turbidity and natural organics removal less efficient in some instances (Rand and Petrocelli, 1985). Aquatic toxicology tests (bioassays) or toxicity tests are used to provide qualitative and quantitative data on adverse (deleterious) effects of toxicant on organisms. Toxicity tests can be used to assess the potential for damage to an aquatic environment and provide a database that can be used to assess the risk associated in a situation for a specific toxicant (Tothill and Turner, 1996).

Environmental bioassays are one of the useful methods for assessing the presence of potentially harmful compounds. *Daphnia* are excellent organisms to be used in bioassays because they are sensitive to changes in water chemistry and are simple and inexpensive to rise in an aquarium. The majority of the bioassays are carried out with the assumption that the test organisms are surrogates for the larger body of organisms comprising natural ecosystems. Bioassays are intended to predict harm or no harm after exposure of living organisms to certain concentrations of a chemical (or mixture of chemicals) periods. They are not reactive in the sense of documenting harm after it is done, which is much better accomplished by in situ surveys (Cairns, 1982). Application of any unconventional nanopolymer as coagulant aid may result in generating byproducts, which

might be harmful or toxic to aquatic life. Therefore, monitoring their toxicity is a crucial task before any decision making on large-scale application. Hence, the aim of this study was to assess the potential toxicity of pamam dendrimers used as coagulant aid in water treatment under different operational conditions.

MATERIALS AND METHODS

The *Daphnia* death rates were measured at different concentrations of PAMAM G2 as coagulant aid in water treatment in order to evaluate its toxicity. At each concentration, the dead toll was recorded after a specified period.

Culture water: *Daphnia* are quite sensitive to the chemistry of the water in which they live. In order to provide standardized culture water, we used distilled water containing essential minerals and nutrients (Table 1). *Daphnia* were brought from the Microbiology Laboratory of Tehran University of Medical Sciences, Iran. For mass cultivation and production of identical genetic traits, sensitive to the toxic material, one of them was isolated and cultivated in specific medium. For optimal culture growth, optimal culture conditions were prepared (Table 2). Oxygen level was adjusted using an aquarium air pump and DO meter was applied. The water temperature was kept at 20°C using an automatic heater and aquarium thermometer; the medium pH was measured using pH meter. For determining hardness, titration method was applied.

Mass production: In order to mass production, one of the young and healthy *Daphnia* was separated from others and was cultured in culture vessel containing specific culture media described in Table 1 and 2. The use of Diluted Mineral Water (DMW) for culturing and testing was widespread due to the ease of preparation. An ordinary 4 L glass beaker was used as culture vessel and was filled with approximately 3 L medium (reconstituted water). Initially, culture vessel was washed well. After the culture was established, each chamber was cleaned weekly with distilled or deionized water and was wiped with a clean sponge to rid the vessel of accumulated food and dead *Daphnia*. The medium in each stock culture vessel was replaced three times each week with fresh medium (Jonczyk and Gilron, 2005; USEPA., 2013).

Feeding: In order to maintain the organisms in optimal conditions and to achieve maximum reproduction, they were feed three times a week.

Jar testing: The toxicity of PAMAM G2 as coagulant aid was evaluated through performing coagulation, flocculation and sedimentation for raw water using ferric chloride as coagulant with

Table 1: Characteristics of the distilled water composition

Compounds	Concentration (g L ⁻¹)
NaHCO ₃	0.192
CaSO ₄ ·2H ₂ O	0.120
MgSO ₄	0.120
KCl	0.008

Table 2: Optimal culture conditions

Factors	Optimal range
pH	7-8.6
Temperature (°C)	20-25
Dissolved oxygen (mg L ⁻¹)	>6
Hardness (mg L ⁻¹ as CaCO ₃)	160-180
Lighting cycle	16 h light/8 h dark

optimum dose and PAMAM G2 as coagulant aid (with 0.5, 1.0, 1.5, 2.0, 2.5 and 3 mg L⁻¹) in jar test. PAMAM G2 was synthesized at color research institute of Tehran. After the process of coagulation, flocculation and sedimentation, the sample was taken from purified water.

Sampling: Six samples were taken after coagulation, flocculation and sedimentation from six glasses that each one has different dose (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L⁻¹) plus one sample as testament for which duration time was 48 h. The bioassay testing was performed in vessel testing in which temperature, dissolved oxygen and pH were measured and controlled. In order to survey the toxicity of each sample during the bioassay, the mortality of *Daphnia* at time intervals of 2, 4, 8, 12, 24 and 48 h was investigated. After this duration, all dead *Daphnia* from all vessels were measured and LC₅₀ was reported.

RESULTS

The toxicity of PAMAM G2 as coagulant aid in water treatment was evaluated. After duration time of experiment (48 h), all *Daphnia* from six vessels plus one vessel as testament were observed and counted; no dead *Daphnia* was observed. Despite several reports about toxicity of dendrimers in different fields, they were not toxic as coagulants aid in water treatment with permitted dose because of interaction them in proses of coagulation, flocculation and sedimentation (Table 3).

DISCUSSION

Nanopolymers have different toxicity due to the functional groups attached or dendrimer generation, for example, different generation of pamam have different toxicity and their toxicity differs based on core and surface groups. PAMAM G2 as nanopolymer plays two roles in water treatment. The first role is as coagulant because of the interaction of surface positive active group with negative particles and neutralizing them and reaction with other toxic cations and anions in water and water purification is considered. The second role is as coagulant aid as other polymers in water treatment (Malik *et al.*, 2000). The results obtained from this test showed that the application of PAMAM nanopolymer as a coagulant aid in water treatment would not lead to toxicity of water. Despite the studies reported that PAMAM dendrimers cause rupturing of red blood cells, or hemolysis, initial studies on PAMAM toxicity showed that PAMAM was less toxic (in some cases, much less) than related dendrimers (Haensler and Szoka Jr., 1993).

In the present research work, the use of pamam as a coagulant aid in water treatment was investigated through different mechanisms such as.

Polymer adsorption: If there is some affinity between polymer segments and a particle surface, then adsorption of polymer chains may occur. The adsorption affinity must be sufficient to outweigh the loss of entropy associated with polymer adsorption, since an adsorbed chain will have a more restricted configuration than a random coil in free solution. Actually, the affinity between a

Table 3: Counting of the immobile ore death *Daphnia* in the test

PAMAM G2 concentration (mg L ⁻¹)	No. of immobile and dead <i>Daphnia</i>	No. of living <i>Daphnia</i>	LC ₅₀ (%)
0.0 (blank)	0	10	0
0.5	0	10	0
1.0	0	10	0
1.5	0	10	0
2.0	0	10	0
2.5	0	10	0
3.0	0	10	0

polymer segment and a surface site need not be great, since there are many attachment points. For a long polymer chain, the chance of all attached segments becoming detached simultaneously is very remote. For this reason, it must be stressed that this is an equilibrium arrangement, which may take some time to achieve after the first contact of the polymer coil with the surface. The rate at which equilibrium is attained is difficult to assess, but times of the order of several seconds for long-chain polymers may be reasonable. This is a very important factor in the kinetics of flocculation by polymers. The extent of the tails and loops and hence the effective thickness of the adsorbed polymer layer, depends greatly on the interaction of polymer segments with the solvent (water) and with the surface. Generally, if the interactions with the surface are weak, segments of the adsorbed chain extend further into the solution. With polyelectrolytes, there can be important ionic strength effects (Dahlgren, 1994).

Polymer bridging: The small flocs produced by coagulation with metal salts can be built up into larger agglomerates by subsequent treatment with a polymer, to form larger particles that have more rapid rates of sedimentation. This involves polymer bridging, in which polyelectrolyte bound to a floc particle has looped and dangling chains that can attach to further particles nearby. Long chain polymers that do not have a high level of charge give best results. One polymer chain adsorbs on two or more particles via an electrostatic or non-electrostatic driving force (Kusakabe and Yoshikuni, 1995; Bolto, 1995). It takes place when the surface is not completely covered, as in the initial stages of the process or when the polymer dose is low. It occurs immediately after addition of the polymer and is very dependent on mixing conditions (Hoogeveen *et al.*, 1996).

Charge neutralization: Charge neutralization takes place at low and high surface coverage, depending on the charges on the polymer and on the surface. Here the polymer has a passive role, merely acting as a multivalent ion, so the process is strictly coagulation (Hoogeveen *et al.*, 1996). Polymer characteristics that favour charge neutralization are substantial doses of a high CD, low MW polymer. Thus, in jar tests on synthetic and natural water of low turbidity and moderate to high colour, high CD cationic polymers are effective in removing the organics responsible for colour and in lowering the production of trihalomethanes after chlorination (Collins *et al.*, 1986).

It is well known that electrostatic interaction gives strong adsorption in these systems and that neutralization of the particle surface and even charge reversal can occur. There is thus the possibility that flocculation could occur simply because of the reduced surface charge of the particles and hence a decreased electrical repulsion between them (Hahn and Klute, 1990; Yoon and Deng, 2004).

Flocculation: The rate of flocculation depends on the square of the particle concentration. Thus, at high solids concentrations, flocculation rates become very high. It is likely that most practical applications of bridging flocculation involve this effect to some extent. So, theories of bridging flocculation assuming equilibrium conditions (Yoon and Deng, 2004) are probably of limited use in practice. In the case of cationic polyelectrolytes and negative particles bridging may occur during the non-equilibrium phase, but electrostatic patch flocculation would be more likely when the adsorbed chains are in a flatter configuration. The important conclusion from this discussion is that bridging flocculation is more likely in rather concentrated suspensions (Runkana *et al.*, 2006).

CONCLUSION

Acute toxicity of PAMAM G2 as coagulant aid in water treatment was investigated by using *Daphnia magna* in bioassay testing. PAMAM G2 was used in extreme permission dosage as coagulant aid. The results showed that PAMAM G2 is not toxic in water treatment.

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