# SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF COLLOIDAL SILVER NANOPARTICLES ENCAPSULATED IN LOW GENERATION POLY(AMIDO)AMINE DENDRIMERS

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*For my beloved parents,*
*Hamidon Bin Sapee and Rasidah Abd Lah*
*My beloved sisters,*
*Nurul Faziha and Nurul Hamimzah*
*My supportive brother in laws,*
*Mohd Khairi and Muhammad Safuan*
*My Fiancee,*
*Muhammad Hafidz Bin Hamidi*

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# ABSTRACT

Poly(amidoamine) (PAMAM) dendrimers of low generations 0.5, 1.0, 1.5, and 2.0 were synthesized in methanol via the divergent synthesis method, which includes alternating reiteration of Michael addition followed by ester amidation starting from ethylenediamine and methyl acrylate. PAMAM dendrimer encapsulated with silver nanoparticles were prepared by *in situ* reduction of $AgNO_3$ solution with $NaBH_4$ in the presence of PAMAM dendrimers. This procedure resulted in stable yellowish colloidal solutions which turn into dark brown colloid and precipitated as deep black solid silver(I) oxide, $Ag_2O$ upon exposure to light. Verification of the PAMAM structures was done using fourier transform infrared (FT-IR) spectroscopy, and $^1H$ nuclear magnetic resonance (NMR) spectroscopy. The results of $^1H$ NMR spectra of synthesized PAMAM indicated that some structural defects were present in the form of missing –NH groups presumably due to incomplete amidation or intramolecular cyclization of terminal amino groups. Characterization of PAMAM-silver nanocomposites by ultra-violet visible (UV-Vis) spectroscopy showed that the surface plasmon resonance (SPR) of colloidal silver shifted from approximately 414 nm to higher wavelengths suggesting specific interaction between PAMAM and silver nanoparticles. This is supported by the fluorescence spectra of generation 2.0 PAMAM-silver nanocomposite which exhibited quenching of the emission peaks of fluorophore at 510 nm after silver encapsulation. The particle sizes of colloidal silver at concentrations of 200 and 800 ppm were found between 5 and 20 nm as determined from transmission electron microscopy (TEM). In this study, it was demonstrated that low generation of PAMAM dendrimers able to act as template to pre-organize silver ions following *in situ* reduction with sodium borohydride ($NaBH_4$). PAMAM dendrimer of generation 2.0 was screened for its potential antibacterial activity against two bacteria, viz, *Escherichia coli* ATCC 11229 (Gram negative) and *Staphylococcus aureus* ATCC 6538 (Gram positive) using disk diffusion technique (DDT) and minimum inhibition concentration (MIC) method. Study on the effect of ratios of silver concentration to PAMAM generation 2.0 indicated that the antibacterial activity against both Gram negative and Gram positive bacteria was lower than for PAMAM-silver nanocomposite. The findings also indicate that the presence of silver in the dendrimer has further enhanced the antibacterial activity against Gram negative bacteria which was also dependent on the concentration of silver solutions. The higher antibacterial activity of PAMAM-silver nanocomposite could be due to strong interaction between negatively charged bacterial cell wall and the cationic PAMAM dendrimer, which possibly decrease the distance between silver nanoparticles and the bacteria. This interaction then enables the silver nanoparticles easily attached to the bacterial cell surface and even penetrate the cell walls, killing the bacteria resulting in the antimicrobial activity.

# ABSTRAK

Dendrimer poli(amidoamina) (PAMAM) bergenerasi rendah 0.5, 1.0, 1.5, dan 2.0 telah disintesis dalam metanol melalui kaedah sintesis divergen yang meliputi pengulangan berselang seli penambahan Michael diikuti pengamidaan ester bermula daripada etilenadiamina dan metil akrilat. Dendrimer PAMAM terkapsul nanopartikel argentum telah disediakan secara penurunan *in situ* larutan akueus $AgNO_3$ menggunakan $NaBH_4$ dengan kehadiran dendrimer PAMAM. Prosedur tersebut menghasilkan larutan koloid kekuningan yang stabil membentuk koloid coklat tua dan mendakan hitam argentum(I) oksida, $Ag_2O$ terbentuk selepas pendedahan kepada cahaya. Verifikasi struktur PAMAM dendrimer telah dilakukan menggunakan spektroskopi inframerah transfromasi fourier (FT-IR) dan spektroskopi resonans magnet nukleus (NMR) spektrum $^1H$. Keputusan spektrum $^1H$ NMR untuk PAMAM yang disintesis menunjukkan beberapa kecacatan struktur wujud dalam bentuk kehilangan kumpulan -NH mungkin akibat disebabkan pengamidan yang tidak lengkap atau pembentukan gelang intramolekul kumpulan amino hujung. Pencirian nanokomposit PAMAM-argentum menggunakan spektroskopi ultralembayung-nampak menunjukkan resonans dapat dijelaskan plasmon permukaan (SPR) bagi koloid argentum beranjak dari sekitar 414 nm ke nombor gelombang yang lebih tinggi mencadangkan interaksi di antara PAMAM dan nanopartikel argentum. Ini disokong oleh spektrum pendafluor nanokomposit PAMAM generasi 2.0-argentum yang menunjukkan pelindap kejutan puncak pancaran fluorofor selepas pengkapsulan argentum. Saiz zarah argentum koloid pada kepekatan 200 dan 800 ppm didapati antara 5 hingga 20 nm seperti yang ditentukan oleh mikroskopi penghantaran elektron (TEM). Dalam kajian ini, telah ditunjukkan bahawa dendrimer bergenerasi rendah mampu bertindak sebagai templat untuk penyebaran ion argentum selepas penurunan *in situ* menggunakan natrium borohidrida ($NaBH_4$). PAMAM generasi 2.0 telah diuji potensi aktiviti antibakteria terhadap dua bakteria, *Escherichia coli* ATCC 11229 (Gram negatif) dan *Staphylococcus aureus* ATCC 6538 (Gram positif) menggunakan teknik resapan cakera (DDT) dan kepekatan perencatan minimum (MIC). Kajian tetap terhadap kesan nisbah kepekatan argentum kepada PAMAM generasi 2.0 menunjukkan aktiviti antibakteria terhadap kedua bakteria Gram negatif dan Gram positif adalah rendah berbanding nanokomposit PAMAM-argentum. Keputusan kajian juga menunjukkan kehadiran argentum meningkatkan lagi aktiviti antibakteria terhadap bakteria Gram negatif yang turut bergantung kepada kepekatan larutan argentum. Aktiviti antibakteria yang lebih tinggi bagi nanokomposit PAMAM-argentummungkin disebabkan oleh interaksi yang kuat antara dinding bakteria yang bercas negatif dan kation dendrimer PAMAM, yang mungkin mendekatkan jarak antara nanopartikel argentum dan bakteria. Interaksi membolehkan nanopartikel argentum melekat pada permukaan bakteria dan meresap masuk ke dalam dinding sel dan seterusnya membunuh bakteria tersebut dan menghasilkan aktiviti antibakteria.

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# LIST OF ABBREVIATIONS

MA - Methyl Acrylate

EDA - Ethylenediamine

MA - Methyl acylate

$\text{ESBL}_S$ - Extended spectrum $\beta$-lactamases

DENs-P - Dendrimer encapsulated nanoparticles

PAMAM - Poly(amido)amine

PPI - Polypropyleneimine

$NaBH_4$ - Sodium borohyride

Ag - Silver

Au - Gold

NPs - Nanoparticles

$AgNO_3$ - Silver Nitrate

AuNPs - Gold Nanoparticles

$\text{AgNP}_S$ - Silver Nanoparticles

DDT - Disk Diffusion technique

MIC - Minimum Inhibition Concentration

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

PRB - Plasma Resonance Band

NA - Nutrient Agar

NB - Nutrient broth

LB - Luria-bertani

a.u - arbitrary unit

gram - gram

μL - microliter

mL - millilitre

dH$_2$0 - distilled water

ppm - part per million

nm - nanometre

rpm - revolution per minute

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# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Dendrimer was discovered in the early 1980's by Donald Tomalia and his co-workers and was known as hyperbranched polymers (Tomalia *et al.,* 1985). It has the unique regular composition and usually in spherical or globular shape (Newkome *et al.*, 1986). The term 'dendrimer' was derived from the word *dendrons*, which is in Greek meaning a "tree" or "branch" and "meros" meaning "part" (Tomalia *et al.*, 2002). Dendron is the term used about dendritic wedge without a core; the dendrimer can be prepared from assembling two or more dendrons. It is also known as arborols, cascade, cauliflower or starburst polymers creating interesting attention due to their attractive properties such as high degree of branching units, high density of surface functional groups, nanoscale size, highly branched three-dimensional architecture, well-defined globular structure and low dispersity make them suitable as new scaffolds for various applications such as energy harvesting, host-guest chemistry and catalysis (Tomalia *et al.,* 1990)(Newkome *et al.*, 2001).

The usefulness and applications of dendrimers towards organic, catalysis, biosciences, polymer science, clinical studies and nanotechnology have constantly increased and especially, dendrimer encapsulated nanoparticles (DENs-P) was actively utilized in the field of chemical and materials research. For example, drug delivery using dendrimer as nanocapsules or immunoreceptors has captured the attention of the medical field (Kesharwani *et al.*, 2014). Dendrimers have already been in various scientific established to be safer, precise and more effective element in the practice of medicine (Newkome *et al.*, 2001).

Dendrimers can be differentiated from conventional synthetic polymers by two fundamentals ways. First, rather than producing linear polymers which is AB monomers, they build from AB$_n$ monomers where $n$ is usually 2 or 3, hence, hyperbranched structures are created. Secondly, they are synthesized by iterative way, which is stepwise synthetic growth where the number of monomer units incorporated in each double (AB$_2$) or triples (AB$_3$) in the previous cycle are combined, leading to nonlinear polymers. A basic characteristic of dendrimer molecules is their layered composition-known as “cascades” or “generations” (Zeng and Zimmerman, 1997). The number of generation is equivalent to the number of cycle repetition that is performed by counting the number of repeating units between the core and the terminal unit.

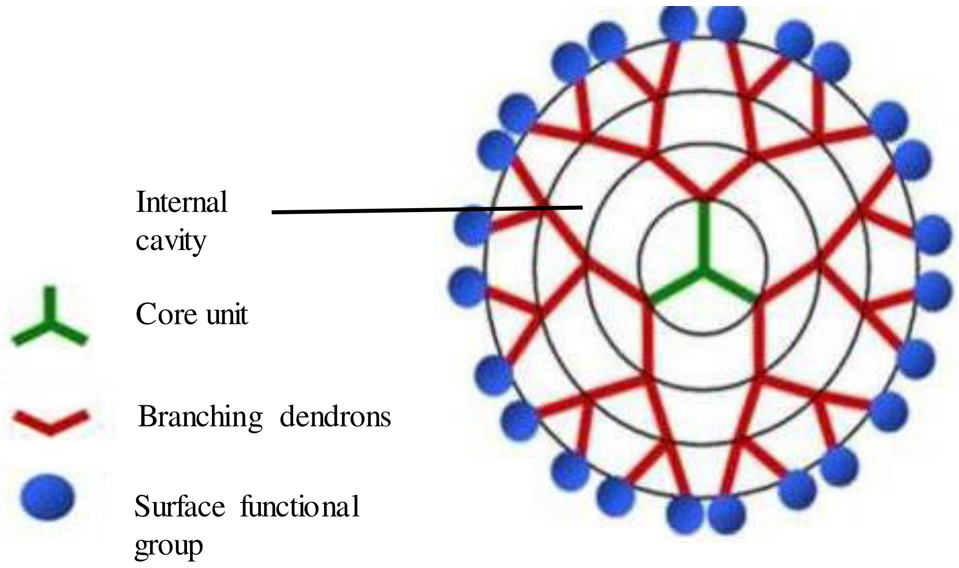

**Figure 1.1:** Structure of basic dendrimer

The structure of basic dendrimer component is shown in Figure 1.1. Generally, dendrimers consists of three main structural components: i.e an interior core, repeated branching unit attached to the initiator core (generations) and surface functional groups (periphery). It also has many end moieties that can be bonded with other functional groups. Hence, because of the size and structures that is highly controllable, their surface can be easily modified. The outer surface represents molecular features such as solubility where the terminal groups are located, and its interface with the surrounding medium. When a hydrophilic substituent is introduced into the surface (the periphery) the dendrimer becomes hydrophilic and water soluble. The interior of these water-soluble dendrimers gives a hydrophobic environment to the incorporated organic molecule. Having a hydrophobic interior and a hydrophilic surface makes dendrimers potentially very useful for drug delivery carrier (Zimmerman and Kim, 1998). Furthermore, dendrimers can be encapsulated by small molecules and nanoparticles and were stabilized due to their interiors. Two commercially available dendrimers are polyamidoamide (PAMAM) dendrimers and polypropylene imine (PPI) dendrimers (Zeng and Zimmerman, 1997).

Dendrimers can be prepared using two pathways, namely the divergent synthesis and the convergent synthesis. Divergent synthesis is the only capable way to produce mass quantities of higher generation dendrimers which are PAMAM and PPI. On the other hand, synthesizing dendrimers via the convergent synthesis will aid in minimizing structural defect (Hawker and Fréchet, 1990). The divergent synthesis was introduced by Tomalia and Newkome, whereby it starts from the central core and extends toward the surface (Tomalia, 1994), (Newkome *et al.*, 1985), while the second synthesis for synthesizing dendrimers is via convergent synthesis which is pioneered by Fréchet, Miller and Moore (Fréchet and Hawker, 1990), (Miller and Neenan, 1990), (Moore and Xu, 1993). As opposed to the divergent synthesis, these dendrimers are building from the periphery toward the central core. Using divergent way to synthesize dendrimers may cause incomplete reactions, usually at higher generations. Whereas for the convergent methods, incomplete reactions can be avoided if the dendritic wedges are separately synthesized. Both of these methods are platforms for the synthesis of dendrimers (Chase and Koten, 2007).

Previous researches have shown that the size, stability and solubility of nanoparticles ranging in diameter from less than 1nm up to 4 or 5 nm can be controlled by using dendrimers acting as templates (Buhleier *et al.,* 1978 and Bosman *et al.*, 1999). There are many potential applications for dendrimers as hosts for catalytically active metal nanoparticles and drug delivery which arise from their chemical and biological properties such as low cytotoxicity and solubility. These properties make dendrimers encapsulated nanoparticles suitable for biomedical and industrial applications. Examples of applications are drug catalytic, optical, magnetic, electrical and biomedical field (Newkome *et al.*, 1999)(Astruc and Charcdac, 2001).

Due to the large size and of number of end group functionality of dendrimers, it is possible for the guest molecules to encapsulate in the interior part or periphery of the dendrimer (Gupta *et al.*, 2006) Hence, it provide opportunity for metal ions to penetrate into interior part or periphery of the dendrimers to form metal containing dendritic system (Zhao *et al.*,1999). Silver has been recognized as a substance with antiseptic properties. In both its metal and ionic form, silver exhibits strong microbial properties towards a broad range of microorganisms (Zhiquan *et al.*, 2012). The antibacterial function of silver nanoparticles is similar to that of silver ions (Mahapatra and Karak, 2008). As a result, a growing interest in the synthesis and study of substances containing silver has developed. One promising nanoparticles material poly(amido)amine (PAMAM) dendrimers is an excellent template for metals and it has attracted attention because of its potential for a wide range of biomedical and industrial applications (Sfand and Tomalia, 2001).

## 1.2 Problem Statement

Recently, general demands for hygiene in daily life have captured attention globally. Inorganic nanoparticles with antimicrobial activity are rising as a new class of biomedical materials to fulfill those needs. It is well known fact that silver ions and silver based compounds are highly toxic to microorganism (Zhao *et al.*, 1998).

This aspect of silver makes it as an excellent choice for bactericidal applications. Toxicity from silver is observed in the form of arygia, only when there is a large open wound and large amount of silver ions are used for dressing. Silver nanoparticles that have been used widely in bactericidal fields are suggested to be non-toxic and applicable towards bacterial applications (Jain and Pradeep, 2005). When silver nanoparticles are used, there is a high increase in the surface are available for the microbe to be exposed to. Due to their high antimicrobial activity, silver nanoparticles is recognized by their strong toxicity towards various bacteria cells where they can interact with the functional groups on the bacteria cell surface and subsequently, inactivate the bacteria (Ray *et al.*, 2007). Besides, their antibacterial activity and immediate antibacterial effect against a wide range of drug-resistant bacteria, silver nanoparticles have particularly characteristics provided by the silver itself. This noble metal tends to induce low bacterial resistance and has low toxicity (Ip *et al.*, 2006).

However, metal nanoparticles tend to aggregate and separate from the solutions and decreasing the antibacterial efficiency. Silver nanoparticles should be size-tunable and colloidal stable under different conditions still remains a great challenge. Hence, it is of great importance to prepare colloidal metal nanoparticles that have long dispersion stability and good antimicrobial efficiency. In order to avoid particle aggregation, low generations of PAMAM dendrimer have been utilized in this study as protective agents to stabilize silver nanoparticles. Low generations of dendrimers tend to exist as relative open forms providing reactive sites on the periphery of the dendrimer. Thus, in theory, the preparation of metal nanoparticles formed inside the early generation dendrimer is more difficult than it is later generations of dendrimers. Despite this potential drawback, low generations of dendrimers have many advantages, such as being easy to synthesize, making them worth preparing.

Dendrimer are known as a new kind stabilizer to produce colloidal metal nanoparticles. It is widely used as drug carriers, self-assembly precursors due to their quasi spherical branched architecture and special solubility properties (Gao and Yan, 2004). For PAMAM dendrimers, toxicity has been shown to be generation dependant with the generation 5 and generation 7, higher generations exhibit increasing toxicity over low generations (Roberts *et al.*, 1996). Moreover, using high generation dendrimer may cause incomplete reactions due to steric hindrance and difficult to modify the terminal groups (Chase and Koten, 2007). It can also lead to high costing, time and stability of the dendrimer and low percentage yield as generation of dendrimer increased.

A study from Malaysia reported that the emergence of *E.coli* and extended spectrum $\beta$-lactamases (ESBL$_S$) that produce multidrug resistant poses antibiotic management problems. ESBL$_S$ enzymes produced by Gram-negative bacilli that commonly recognized in *P.aeruginosa* (Tenover *et al.*, 2003). The emergence of ESBL$_S$ with multiple resistant creates a serious problem in hospital setting. The widespread of this application can lead to the increase in antibiotic resistance in bacterial pathogens (Kang *et al.*, 2005). Infections caused by drug-resistant microorganisms resulting increases of death, disease and cost related to prolonged treatments. The increasing number and occurrence of antibiotic resistant bacterial strains has captured interest in the use of silver as an antibacterial agent (Stobie *et al.*, 2008) (Pissuwan, *et al.*, 2008).

PAMAM dendrimers are water soluble substances and are considered to be critical for biological applications (Tomalia *et al.*, 2005). It also has tendency to form cationic structures under physiological conditions. The primary amines on the surface of the dendrimer become readily protonate, thus create a polycationic dendrimer with ammonium terminal groups increasing alkalinity of a solution. The unique chemical and biochemical properties of PAMAM dendrimers have encouraged us to utilize PAMAM dendrimer as template for applicable as a bactericide with minimum toxicity. The goal of this study was to synthesize low generation PAMAM dendrimer G-2.0 encapsulated silver nanoparticles and to investigate antibacterial activity and potential for its biological application.

## 1.3 Research Objectives

The main objectives of this research are:

i. To synthesize low generation PAMAM dendrimers of generation 0.5, 1.0, 1.5, and 2.0 by divergent synthesis method.
ii. To synthesize PAMAM dendrimer encapsulated colloidal silver nanoparticles by reduction of $AgNO_3$ with sodium borohydride.
iii. To investigate the antibacterial activity of PAMAM-encapsulated with silver nanoparticles in aqueous solutions at different concentrations against *Escherichia coli* (Gram negative*) and Staphylococcus aureus* (Gram positive).

## 1.4 Scope of Study

The scope of this study was firstly the synthesis of low generation PAMAM dendrimers (generation 0.5, 1.0, 1.5 and 2.0) *via* Michael Addition using the divergent synthesis. PAMAM dendrimer was characterized using a number of characterization techniques such as Fourier Transform Infrared (FT-IR) spectroscopy and $^1$H NMR spectroscopy.

Secondly, PAMAM dendrimer encapsulated silver nanoparticles was synthesized via chemical reduction of silver nitrate ($AgNO_3$) by using reducing agent, sodium borohydride ($NaBH_4$). After the encapsulation with silver nanoparticles, Ultraviolet-Visible (UV-Vis) Spectroscopy, Fluorescence spectroscopy and Transmission electron microscopy (TEM) were used to observe the chemical properties.

Lastly, antibacterial activity of silver nanoparticles at different concentrations of silver (200 ppm, 400 ppm, 600 ppm and 800 ppm) with PAMAM dendrimer were investigated against *Escherichia coli* (Gram negative*) and Staphylococcus aureus* (Gram positive), through Disk Diffusion Technique (DDT) and Minimum Inhibition Concentration (MIC) technique.

## 1.5 Significance of the research

The results of this research contribute towards the biological applications of PAMAM dendrimer silver as the effective antibacterial agent for Gram positive bacteria in comparison with Gram negative bacteria. It also contributes towards green chemistry since the research was done using water and at ambient temperature. Hence, hyperbranched polymers, PAMAM act as stabilizer to control the size and stability of the metal nanoparticles.

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