Deanship of Graduate Studies

Al-Quds University

Dendrimer-induced DNA bending

Jannat Jihad Salim Al Shawwa

M.Sc. Thesis

Jerusalem – Palestine

1436 / 2015

Dendrimer-induced DNA bending

Prepared by:

Jannat Jihad Salim Al Shawwa

B.Sc. Chemistry/Science technology. Al-Quds University-Palestine

Supervisor: Dr. Khawla Qamhieh

A thesis Submitted in Partial fulfillment of requirements for the degree of Master of Applied &Industrial Technology, Al- Quds University.

1436 / 2015

Al-Quds University

Deanship of Graduate Studies

Applied and Industrial Technology Program

Thesis Approval

Dendrimer-induced DNA bending

Prepared by: Jannat Jihad Salim Al Shawwa

Registration No.: 21210286

Supervisor: Dr. Khawla Qamhieh

Master thesis submitted and accepted, Date: 10/5/2015

The names and signatures of the examining committee members are as follows:

1-Head of Committee: Dr. Khawla Qamhieh Signature

2-Internal Examiner: Dr. Mohammed Abu al-Haj Signature

3-External Examiner: Dr. Jamal Ghabboun Signature

Jerusalem – Palestine

1436 / 2015

Dedication

I Dedicate my modest work to my family. A grateful feeling of graduation to my loving parents

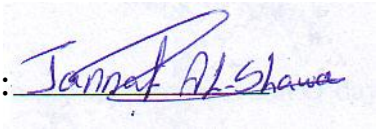
JIHAD & HAJAR

Whose love, encouragement, support

Day and night prayers motivate me to get higher success target.

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research. Materials of works found by other researchers are mentioned by references. This thesis or any part of the same has not been previously submitted for any higher degree.

Signed: 

Jannat Jihad Salim Al Shawwa

Date: 5/10/2015

Acknowledgement

I would like to thank my supervisor Dr. Khawla Qamhieh for her support, encouragement and for providing me this chance to achieve the knowledge and experience in the field of research.

Thanks and appreciation to my committee Dr. Jamal Ghabboun (external examiner)
Dr. Mohammed Abu al-Haj (internal examiner).

Special thanks to my gorgeous husband Fadi for the moral support and for being along with me achieving my master degree dream!

Thanks to my still not born son O'day for his positive inspiration for me at my abroad life towards reaching my goal!

Finally I must thanks my dear family, sisters and brothers, Hanadi, Niveen, Dr. Saleh, Eman, Ibrahim, Hazar and Tasneem. Thank you all.

Abstract

The complex build–up of biomaterials consisting of biopolymers, namely DNA, and soft particles poly amidoamine (PAMAM) dendrimers of ammonia cored of generation (G1, G2, G3, G4, G6) and ethylenediamine cored of generation (G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10) has been studied by using a new developed theoretical model by Qamhieh and coworker describing the interaction between linear polyelectrolyte (LPE) chain and ion-penetrable sphere. Many factors affecting dendrimer/LPE complex have been investigated such as the dendrimer size generation, the Bjerrum length, salt concentration, and rigidity of the LPE chain (Persistence length).

Through the complexation of LPE chain with one dendrimer, it is found that the wrapping degree of the chain around dendrimer increases by increasing dendrimer`s generation, Bjerrum length and salt concentration decreases by increasing the Persistence length and found that the optimal wrapping length of the LPE chain around dendrimer depends on dendrimer generation. Also the effect of 1:1 salt concentration on complexation of DNA plasmids with one dendrimer of different generation has been studied.

The complexes formed between a multiple PAMAM dendrimers and oppositely charged LPE chain depend on the generation and on type of dendrimer cored (ammonia and ethylenediamine cored), it is shown that the optimal wrapping length increases while the linker is decreased. This result is in agreement with previous results of Qamhieh and co-workers, for other generations of dendrimers.

Table of Contents

List of Tables

List of Figures

Abbreviations

DNA	Deoxyribonucleic acid
LPE	Linear Polyelectrolyte
dsDNA	Double strand DNA
ssDNA	Single strand DNA
EDA	Ethylenediamine
bp	Base- pair
l_{opt}	The optimal wrapping length of chain around dendrimer
l_p	The persistence length of LPE chain
l_B	Bjerrum length
Cs	Salt concentration
G	Generation
Cryo-TEM	Cryo-Transmission electron microscopy
TEM	Transmission electron microscopy
MD	Molecular Dynamic
BD	Brownian Dynamic
DLS	Dynamic light scattering
AFM	Atomic Force Microscopy

Chapter One

Introduction

Chapter One

1.1 Introduction

Nanoparticle drug-delivery systems are the popular ones as they are able to increase the selectivity and stability of therapeutic agents. The most common form carries the genetic material of gene therapy involves using Deoxyribonucleic acid (DNA) that controls all functions inside living cells. The unique material properties of DNA have made it an attractive molecule for material scientists and engineers interested in micro- and nano-fabrication.

Gene therapy is one promising and rapidly developing medical approach, which aims to treat genetic diseases by insert a functioning gene into cells to correct a cellular dysfunction or to provide a new cellular function (Culver, 1994). Although important progress has been made in the area of gene therapy, but the main problem in gene therapy is the loss of efficient and safe vectors for gene delivery. In the 1980s, Scientists began to look into gene therapy. However the first clinical study using gene therapy was reported on September 14th, 1990 (Rosenberg et al., 1990). The gene therapy is a hopeful treatment option for a number of diseases that have no other cures, (including inherited disorders, combined immunodeficiency syndromes, muscular dystrophy, hemophilia, certain viral infections and many cancers result from the presence of defective genes)**.**

The challenge and main principle of developing successful gene therapy for any specific condition is to site the delivery of a drug of its action into the human cells to get the needy result in nanomedicine. There are many ways to deliver the gene inside the cell: viral (viruses) and non-viral, Non-viral vectors such as Liposome, Cationic polymer sand

Cationic Dendrimers, are attractive because of their lower immunogenicity, greater safety and easy of preparation. Viral vectors have been used in ~70% of the clinical trials to date (Edelstein et al., 2004).There are certain types of virus being used as carrier for genetic material such as Adenovirus and Retrovirus. However viral have many benefits to carry and connect the gene into a specific target cell and have high transfection efficiency. Nevertheless there are many defects that are related to safety, although they are efficient in delivering a gene to the target cells, toxicity for human body and highly immune response (Itaka and Kataoka,.2009).Therefore and because of this side effect we need to change the viral system to use natural and chemically synthetic polymers such as dendrimers. The first theoretical model of the dendrimer that will be used in our study is polyamidoamine (PAMAM) of different generation.

DNA is vital for its function and genetic information storage (Zinchenko and Chen, 2006). To transfer DNA into gene therapy cells, DNA as polyelectrolytes needs to be compacted with colloids such as dendrimers. The complexes reaction between oppositely charge dendrimer and linear polyelectrolyte (LPE) are a subject of great interest in physics, chemistry, and biology because such reactions are ubiquitous in nature (Maiti and Bagchi, 2006). One hope of the DNA/ dendrimers complexation is to understand the factors effected and control of these complexs such as type and generation of dendrimer, the charge (Ze) of the cationic sphere, the radius (R) of the cationic dendrimer, the ionic strength of the aqueous solution, the linear charge density of the polymer $-e/b$, and the pH of the solution and polymer flexibility. There are several theoretical and experimental studies and computer simulation that prove the rightness of using dendrimers as carrier of DNA and the complexation between DNA and dendrimer.

In 1999 were presented the first theoretical models on sphere – LPE chain complexation . Park et al (1999) showed that by supposing a semiflexible and highly charged PE chain that counterion emission leads to overcharging.

Welch and Muthukumar (2000) first reported the complexation between a model dendrimer (G4-G6) and oppositely charged linear polyelectrolyte under varying pH conditions using Monte Carlo (MC) simulations. They also predicted theoretically, the adsorption criteria depending on the salt concentration, size of the dendrimer, charge density of dendrimer, polyelectrolyte chain and length of the linear polymer and found good agreement with the simulation results.

Nguyen and Shklovskii, (2001) have developed the correlation theory for the overcharging effect in complexes formed by spherical impenetrable macroion and oppositely charged flexible linear polyelectrolyte in salty aqueous solution, They found that with the excess of macroion, the polyelectrolyte becomes consistently overcharged and that the macroion, might come into molecular contact with each other despite their strong mutual electrostatic repulsion (charge inversion), and when increasing the salt concentration, the electrostatic repulsion becomes stronger. The structure and the composition of the complex formed were offered at spherical macroion charges, chain lengths of the polyelectrolyte, and different linear charge densities of the LPE. Nguyen and Shklovskii presented analytical result from Monte Carlo simulations.

Schiessel and co-workers (2001) studied first the case of complexation with a single positively charged hard sphere and calculated the wrapping length of the chain. However he accounted complexes formed between LPE and positively charge sphere.

Lyulin et al (2005) performed Brownian dynamics computer simulations to study structure of complexes formed by charged dendrimers (G1-G4) and longer anionic linear polymer chains of different degree of polymerization (N_{ch}). He showed that when the monomers of the LPE chains equal the number of dendrimer's terminal charged groups located close to these terminal groups that facilitate the chain release. For longer chains the total number of the LPE chain monomers adsorbed onto a dendrimer exceeds the number that is necessary for a dendrimer neutralization, and the overcharging phenomenon is observed.

Maiti and Bagchi (2006) considered the complexation of PAMAM dendrimers of generations (G2-G4) at various protonation and base pair (bp) ssDNA in explicit water by using atomistic MD simulation which it takes 2 ns for the ssDNA to start wrapping around the DNA. At neutral pH, G2, G3, and G4 PAMAM dendrimer have 16, 32, and 64 protonated amines, respectively, while ssDNA has 37 negative charges. They showed for smaller dendrimers like G2 and G3, the charge ratio is not enough to have a complete wrapping of the ssDNA onto the dendrimer, also higher generation dendrimer with a positive and larger size like (G4) have enough positive charge to neutralize the 37 charges on the ssDNA and collapse of ssDNA on the surface of dendrimer to get compact complex then after a while it penetrated inside dendrimer even after 13 ns (see Figure 1.1).

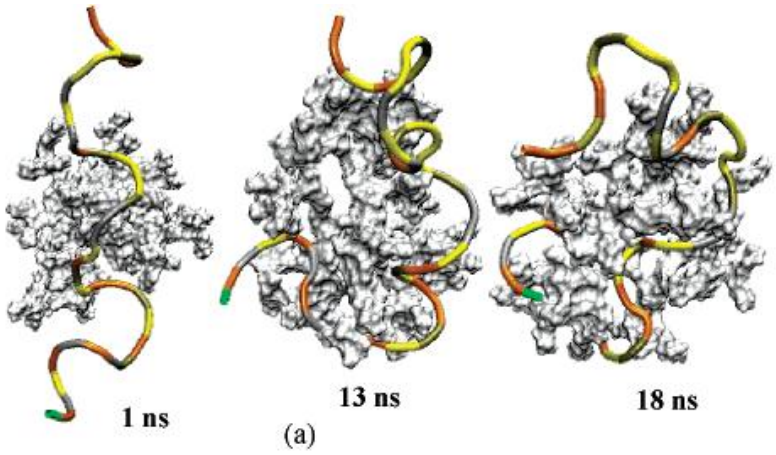


Figure 1.1: Several snapshots in a few ns of formation of DNA-dendrimer complex (Maiti and Bagchi, 2006).

Örberg et al (2007) used dynamic light screening (DLS) and steady state fluorescence to study the interaction between DNA and G4 dendrimers as a function of the charge ratio and proposed a binding model which is cooperative of both DNA and PAMAM dendrimer (see Figure 1.2).

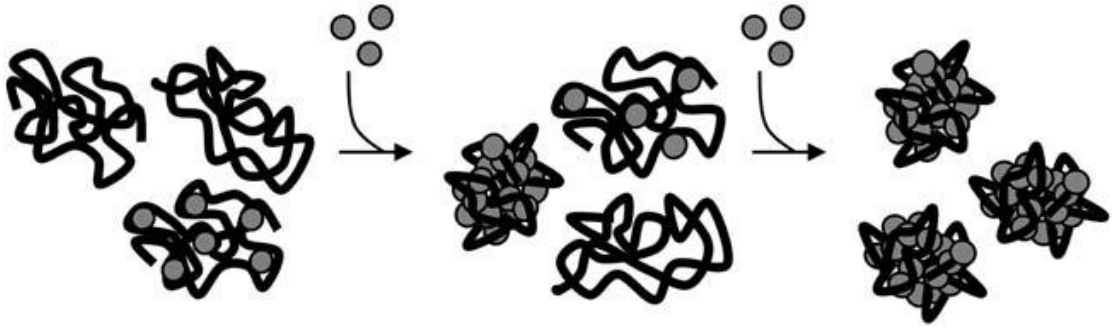


Figure 1.2: Proposed cooperative binding model for discrete aggregates formed between DNA and PAMAM dendrimers at low charge ratios (Örberg et al., 2007).

Ainalem et al (2009) studied the morphology of poly(amido amine) (PAMAM) dendrimer and DNA aggregates depends on the dendrimer generation, using cryo-TEM, dynamic light scattering (DLS) and fluorescence spectroscopy to detect how the size, composition and morphology of aggregates formed between monodisperse DNA sample of 4331 base pairs (bp) and PAMAM dendrimers of generation (G1, G2, G4, G6 and G8). The study showed the smaller sized dendrimers (G1 and G2), which have a lower total charge per molecule, allow the formation of well-structured rods and toroids. In contrast, globular aggregates are formed with higher generation dendrimers. The cooperative nature of the condensation process as cryo-TEM and DLS show that dendrimer/DNA aggregates, containing condensed DNA, coexist with free extended DNA chains. The fluorescence study shows that the number of dendrimers bound per DNA chain decreases with the dendrimer generation but is independent of the charge ratio.

Qamhieh, Nylander, and Ainalem (2009) showed the complexation between a positively charged poly(amido amine) (PAMAM) dendrimers of G4 and DNA, in dsDNA lengths; 2000 basepairs (bp; L= 680 nm) and 4331bp (L= 1472.5 nm) using a theoretical model by Schiessel for a semiflexible polyelectrolyte and hard spheres. The model of dendrimers is to be regarded as soft spheres, which means that the radius was not constant when the complexation between DNA and dendrimer happened. Through this modification of the model they studied how much of the LPE chain wrapped around the dendrimer and the number of dendrimers in the aggregate and the total charge of the complex adapt by dendrimer of G4 complexes with semiflexible LPE chain of two lengths.

Qamhieh and Abu Khaleel (2011) developed a new analytical model describes the complexation of linear polyelectrolyte (LPE) and ion-penetrable spheres, by replacing the hard spheres by soft (penetrable) spheres, in the theoretical model developed by Schiessel et al. throughout the study, they confirmed the effect of the medium's environments on the complexation of LPE chain with one dendrimer such as 1:1 salt concentration on complexation of different generations that has been investigated. They showed also that the wrapping degree of the chain around the dendrimer increases by increasing dendrimer's charge (decreasing pH), Bjerrum length, salt concentration, and decreases by increasing the rigidity of the chain.

Carnerup, Ainalem, Viveka and Nylender (2011) reveal the structure and morphologies of aggregates formed between DNA and PAMAM dendrimers of different generation (G1, G2, G4, G6, and G8) and the effect of the amount of the salt concentration on the condensation by using many techniques such as cryogenic transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS) as well as fluorescent spectroscopy. They observed when the salt concentration increases, the size of aggregate formed by lower generations (G1, G2, and G4) increases, so toroidal aggregates diameter up to several hundreds of nm. For higher generation dendrimers (G6), the size of the condensed DNA aggregates does not change but at high salt concentration rod-like aggregates are observed (see Figure 1.3). Whereas the size and morphology of G8 dendrimers are insensitive to salt concentration. The effective neutralization aggregate happened for higher charge of higher generation.

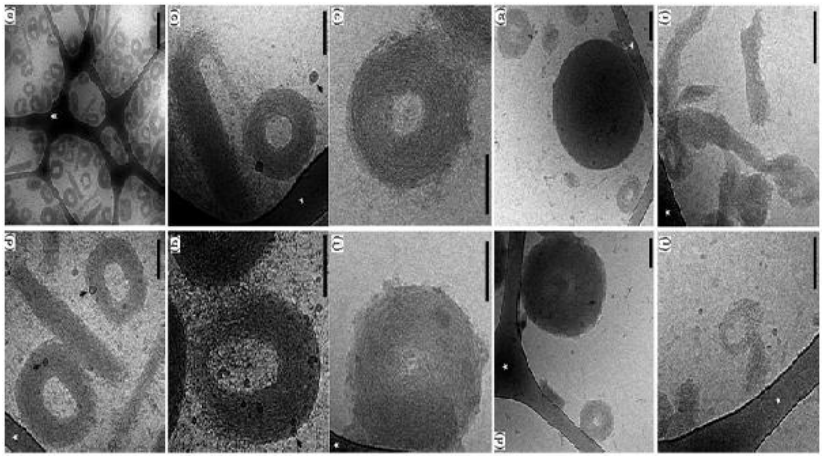


Figure 1.3: Cryo-TEM micrographs of Gx/DNA aggregates condensed in 150 mM NaBr: (a–d) G1/DNA, (e and f) G2/DNA, (g and h) G4/DNA and (i and j) G6/DNA (Carnerup et al., 2011).

Qamhieh and co-workers (Qamhieh et al., 2014) provided the formation and structure of complexes and aggregates composed of DNA (4331 bp) and positively charged PAMAM dendrimers of different generations (G1, G2, G4, G6 and G8). They showed that the number of DNA turns around one dendrimer for low generation (specially G4) form highly ordered rods and toroids for dendrimer/DNA aggregates, while the DNA wraps several turns around the high generation dendrimer display globular structure of complex (see Figure 1.4). The DNA penetration required for the complex to become charge neutral depends on dendrimer generation, where lower generation dendrimers require little penetration to give charge neutral complexes. High generation dendrimers display charge inversion for all considered dendrimer sizes and degrees of penetration. The net-charges of the aggregate have been measured using zeta potential.

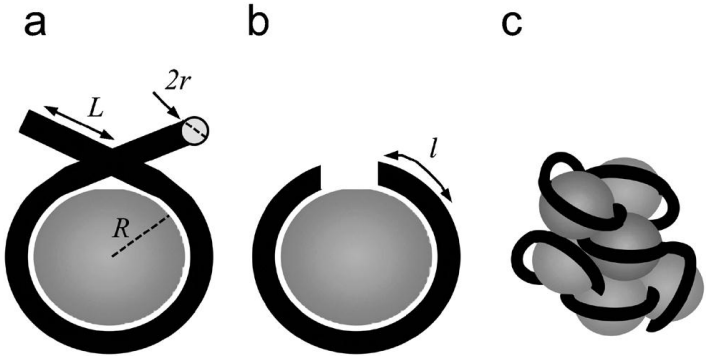


Figure 1.4: Schematic representation of (a) a fraction of a DNA molecule is shown wrapped around one dendrimer. The DNA piece linking to the next dendrimer in an aggregate.(b) A dendrimer/DNA complex consisting only of one dendrimer and the DNA segment actually wrapping the dendrimer. (c) The dendrimer/DNA aggregate consisting of the entire DNA molecule and a multiple of dendrimers (Qamhieh et al., 2014).

1.2 PAMAM Dendrimers

Polyamidoamines (PAMAM) dendrimers are highly branched polymeric molecules, which are characterized by unique three dimensional architecture, highly regular structures, highly monodisperse macromolecules and globular. Dendrimers are a family of nanosized, which is the unique molecular topology that are appropriate for direct control over dendrimer's size, shape, interior density, and surface functionality, is the symmetrical branching structure of dendrimers. This is to allow dendrimers to serve both as nanocontainers and as surface-active colloid particles for a wide range of biomedical and industrial field such as gene and drug delivery. Dendrimers have a positive makes

charge which it possess a great ability to bind with DNA to get inside the cell through the negative cell membrane, and compose dendrimer/DNA complexes.

Dendrimers have often been referred to as the "Polymers of the 21st century". In 1978 the first successful attempt to create and design dendrimer structures by organic synthesis were carried out by Vogtle and coworkers (Buhleir *et al.*, 1978) also naming the structure "cascade molecules". However after this, in the early 1980, Donald Tomalia and his co-workers had worked and synthesized the first family of "dendrimers" was polyamidoamines (PAMAM) originated from the word "dendron", which means "tree" in Greek and "meros", meaning "part" and refers to the special organization of polymer units. At the same time prof. George R. Newkome's group independently reported synthesis of similar macromolecules, they called it "arborols" from the Latin word "arbor" also meaning a tree.

1.2.1 Dendrimers Structure

Dendrimers are just in between molecular chemistry and polymer chemistry. Dendrimers consist of three major architectural components as an initiator core, interior layers branches (generations) composed of repeating units and exterior end groups (terminal functionality) (see Figure1.5). Dendrimers are built from a starting atom (core) which contains two or more functional groups such as nitrogen atom that acts as starting unit. Each carbon and other elements are added to core by a repeating series of chemical reactions that produce a spherical branching structure. As the process unit repeats, successive branch layers are added around the core and the sphere can be expanded to the

size called generation (G).The end groups are the exterior layer that carry the functional group (Pushkar, 2006).The central unit for this group of molecules is either ammonia (NH_3) or ethylendiamine (EDA) with amidoamine as repeated unit. Table 1.1 shows mainly the differences between dendrimers and linear polymers. For PAMAM dendrimer synthesis is observed after tenth generation. The tenth generation PAMAM contains 6141 monomer units and has a diameter of about 124 Å (Tomalia et, al., 1990).

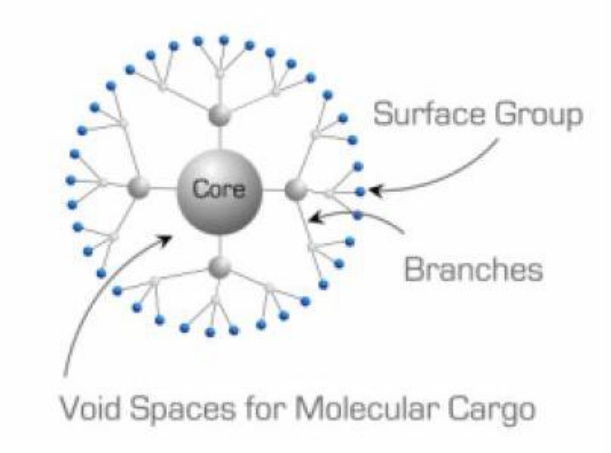


Figure 1.5: Schematic representation of generation two (G2) Dendrimer (Reshama et al, 2012).

1.2.2 Dendrimer's Properties

Dendrimer's molecules are characterized by structural perfection, they are monodisperse macromolecules, unlike linear polymers. The architecture of dendrimer molecule shows some safely improved physical and chemical properties when compared to linear polymers, the properties of dendrimers are dominated by the functional groups on the

molecular surface which is responsible for reactivity and high solubility and miscibility. The solubility of dendrimers is strongly influenced by the nature of surface groups which can be in hydrophilic groups soluble in polar solvents. Dendrimers having hydrophobic end groups are soluble in nonpolar solvents (Klajnert and Maria, 2001).

Dendrimers are highly symmetric and spherical polymer having 5-10 nanometers in diameter with unique a structure whose properties are catching great interest from both scientists and technologists. The performance of these dendrimers is dependent upon its size, generation, surface functional groups. The shape of low generation of dendrimer embrace an open ellipsoidal shape, conversely the higher generation has more spherical shape (Change et.al 2008), so the shape of dendrimer also changes when generation changes. The diameter of the dendrimer increases as the generation of dendrimer increases e.g., ammonia cored dendrimer of generation G1 has a diameter of 1.9nm and G7 has a diameter of 8.8nm (see Figure 1.6) (Change et al., 2008).

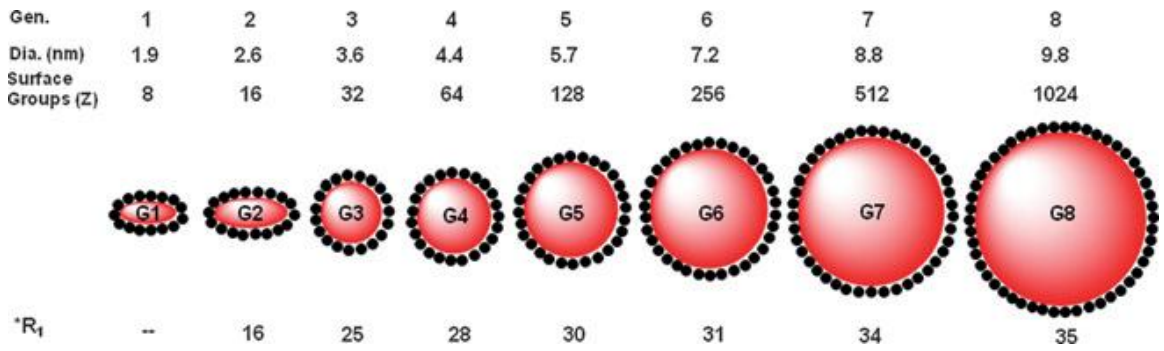


Figure1.6: Type PAMAM dendrimer series at different generation (Tomalia et al., 2010).

The polymerization process in linear polymers is usually random in nature, produces molecules of different size, and can be controlled of molecular mass and the size of

dendrimers during synthesis process**.** 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. Dendrimer solutions have significantly lower viscosity than linear polymers. When the molecular mass of dendrimers increases, their intrinsic viscosity goes through a maximum at the fourth generation (G4) and then begins to decline. Such behavior is unlike that of linear polymers. For classical polymers the intrinsic viscosity increases continuously with molecular mass. The unique properties in dendrimer structure make them best device for using as vectors in gene therapy (see Table 1.2).

Table: 1.1: Properties of Dendrimer and linear polymers (Mishra et al., 2011).

Sr. No.	**Property**	**Dendrimers**	**Linear Polymers**
1	Structure	Compact, Globular	Not compact
2	Synthesis	Careful & stepwise growth	Single step polycondensation
3	Structural control	Very high	Low
4	Architecture	Regular	Irregular
5	Shape	Spherical	Random coil
6	Crystallanity	Non-crystalline, amorphous materials -lower glass temperatures	Semi-crystalline/crystalline materials -Higher glass temperatures
7	Aqueous solubility	High	Low
8	Nonpolar solubility	High	Low
9	Viscosity	Non linear relationship with molecular weight	Linear relation with molecular weight
10	Reactivity	High	Low
11	Compressibility	Low	High
12	Polydispersity	Monodisperse	Polydisperse

1.2.3 Dendrimers Synthesis

The first successful attempt to create and design dendrimers molecules structures by organic synthesis was performed by Vogtle et al. and Buhleier et.al (Vogtle and Buhleier et.al 1978). The synthesis of dendrimer have different parts to control properties such as solubility, flexibility, thermal stability, size, shape and number of branches on the dendrimer. Many dendrimer synthesis depend upon traditional reactions, while others researchers involve the use of modern techniques and chemistry, such as solid-phase synthesis, organosilicon chemistry, organo-phosphorus chemistry, and other modern organic methodologies (Fréchet and Tomalia, 2001). There are two defined methods for dendrimer synthesis, divergent method and a convergent one, there is a major difference between these two construction concepts. However, because the actual reactions consist of many steps needed to protect the active site, it is difficult to synthesize dendrimers using either method.

In 1978 Vögtle made the first divergent synthesis of dendrimers. Divergent dendrimer growth is a technique that effects on dendrimers structure which happening in a stepwise starting from the initiator core molecule. The core molecule reacts with monomer molecules containing one reactive and two dormant groups giving the first generation dendrimer, (see Figure 1.7). This process is repeated a number of times until a building blocks to the surface of dendrimers growing up layer after layer, where the molar mass of the dendrimer is doubled. There are two Problems occurring from side and incomplete reactions of the end groups that lead to structure defects. Firstly, some of branches are shorter than others, which can cause trailing generation. Secondly, such impurities can

influence the functionality and symmetry of the dendrimer, but due to the relative small size difference between perfect dendrimers.

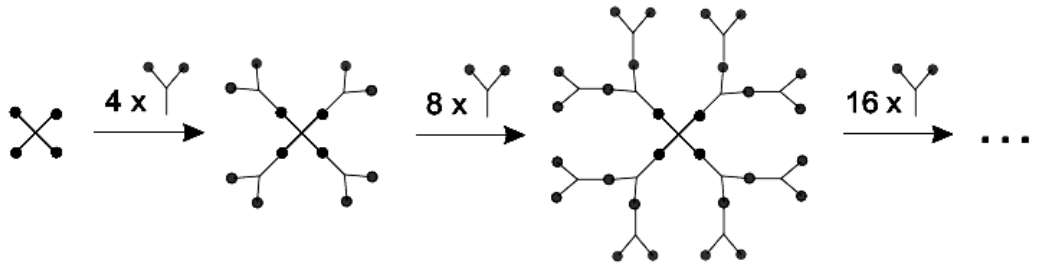


Figure 1.7: Schematic drawing showing the divergent method for synthesis of dendrimers (Klajnert and Maria et al., 2001).

The convergent methods were developed as a response to the weaknesses of the divergent synthesis. In the convergent approach, the dendrimers are built up stepwise from the inwards and end group until attached to a core molecule, when growing wedges are large enough, several are attached to a suitable core to give a complete dendrimer (see Figure 1.8).This method has many advantages, easy to purify the desired product and to form shorter branches along the way, leads to improved monodispersity of the final dendrimers. The convergent approach does not allow the formation of high generations because steric problem occur in the reactions of the dendrons and the core molecules (Asheesh, et al., 2012).

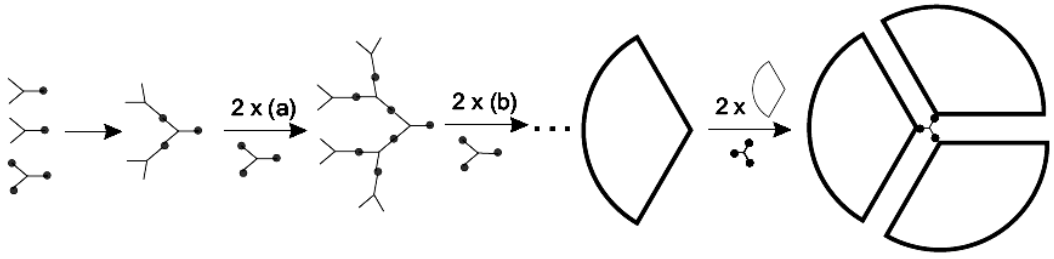


Figure 1.8: Schematic drawing showing the convergent method for synthesis of dendrimers (Klajnert and Maria, 2001).

1.2.4 Dendrimers Applications

Dendrimers have unique molecular architectures and properties that make them attractive and smart polymers for using them as nanocarriers in various applicability in pharmaceutics, industry and diagnosis such as gene delivery, drug delivery, biomedical and other significant industrial applications. Dendrimer-based transfection agents have become routine tools for many molecular and cell biologists but therapeutic delivery of nucleic acids remains a challenge.

The nanoscale size and confession abilities make dendrimers as ideal building blocks for self-organization systems (Fréchet 1994, Zimmerman et al.1996). The matter of efficient and safe delivery is important in the field of gene therapy, and several nanotechnology approaches to packaging nucleic acids.

The dendrimers unique molecular structure is the most promising examples of synthetic molecules with certain great potential as delivery vectors for gene transfection. By a control of the size, structure and the charge density of the dendrimers, high efficiency and functionality of dendrimer/DNA complexes transfection can be produced.

Dendrimers can also mimic biological macromolecules analogous to enzymes, viral protein, antibodies, histones, and polyamines. Dendrimers can function as drug carriers either by encapsulating drugs within the dendritic structure or by interacting with drugs (Tomalia et al.,1985). Modern medicine uses a variety of this material as potential blood substitutes, e.g., polyamidoamine dendrimer (Ruth-Lorella et. al., 2005).

In 2011, Mishra, et al, used different techniques and methods to characterize dendrimers polymer such as X-ray scattering (SAXS), Nuclear Magnetic resonance (NMR), Infra-red (IR) and Fluorescence spectroscopy.

1.3 General characteristic of DNA

The structure of the Deoxyribonucleic acid (DNA) double helix, with its complementary base-pairing, is one of the greatest discoveries in biology in the 20th Century by a German biochemist named Frederich Miescher in 1869. But for many years, researchers did not realize the importance of this molecule. It was not until 1953 that James Watson, Francis Crick, had found the structure of DNA a double helix form B using X-ray diffraction data from fibers of DNA which they realized could carry biological information (see Figure 1.9 a,b).Watson, Crick and Maurice Wilkins were awarded the Nobel Prize in medicine in 1962 for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material.

(a) (b)

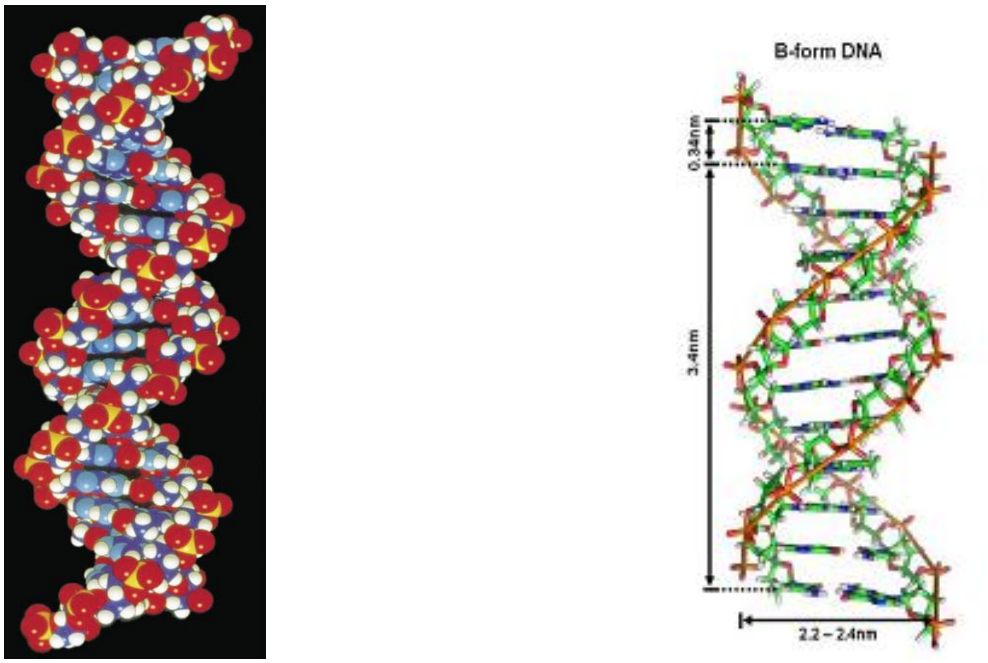


Figure1.9: (a) Space-filling atomic model of the DNA double helix. Coloring: phosphorus yellow; oxygen red; carbon dark blue; nitrogen light blue; hydrogen white, (b) Structure and dimension of B-form DNA (Watson and Crick 1953).

DNA is a genetic material playing a critical part in all living organisms and many viruses, because it is the key molecule responsible for storage, transcription, realization and that carries the genetic information which controls all function inside living cells. DNA is composed of two polynucleotide chains (double-chains) molecule running in opposite directions and twined round one another that forms a double helix, serves as the carrier of genetic information within cells. The double helix structure of DNA consists of four nitrogen bases. These bases are divided into two types (a) Pyrimidines which are six membered heterocyclic includes thymine " T", cytosine "C" (see Figure 1.10a), (b)

Purines differ from Pyrimidines which have two carbon-nitrogen rings, include adenine "A" and guanine "G"(see Figure 1.12 b).In 1953 James Watson and Francis Crick have described that DNA serves as the carrier of genetic information within cells and known thymine (T) cytosine (C) adenine (A) and guanine (G) as base pairing, the order of these bases is what determines DNA's instructions, or genetic code.

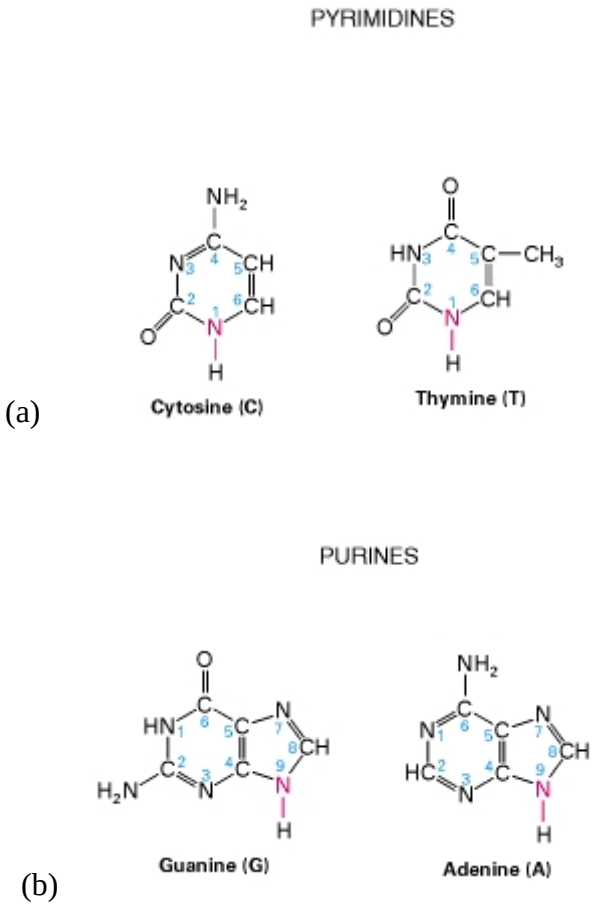
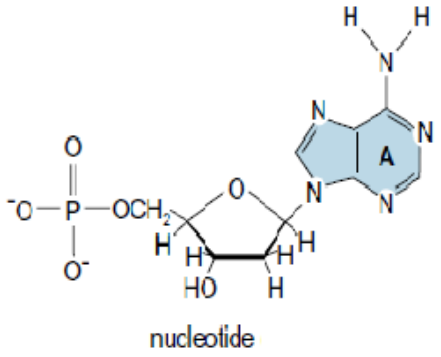


Figure 1.10: The structure of pyrimidines and purines.

The backbone of the double chains (nucleotide) is composed of deoxyribose sugar groups and phosphate groups. The phosphate group carries a negative charge, which makes the

total charge of DNA strand negative. The nucleotides are joined together by hydrogen bonds between the nitrogenous bases, each base being joined to a partner base on the other chain. The sequence of bases of the two complementary chains constitutes the genetic code. The nucleotide consists of a phosphate and five types sugar known 2-deoxyribose, one of nitrogen base is attached from each sugar (see Figure 1.11)**.**

We can describe the DNA as a linear polyelectrolyte (LPE) chain with negatively charged repeating units thus bearing an electrolyte group. Polycations and polyanions are polyelectrolytes. These groups dissociate in aqueous solutions such as water, making the polymers charged.



nucleotide

Figure 1.11: Structure of nucleotide (Franklin and Gosling, 1953).

1.4 Transfection of dendrimer/DNA aggregate in Gene therapy.

The observation that free plasmid DNA is able to transfect the living cells when given in the suitable way, but will normally be degraded in the systemic circulation; provides the set of reasons for complexing of the plasmid DNA. This complexing occurs with the help of a delivery system such as dendrimer which tends to compact and protect the nucleic acid. Furthermore, the delivery system should help to target the therapeutic nucleic acid to the desired site of action. The most common strategy employed for the complexing of DNA is based on electrostatic interaction between the anionic DNA and the positive charges dendrimer of the synthetic vector (see Figure 1.12). In contrast, there are many factors controlling the transfection process such as charge inversion, electrostatic interaction and pH value.

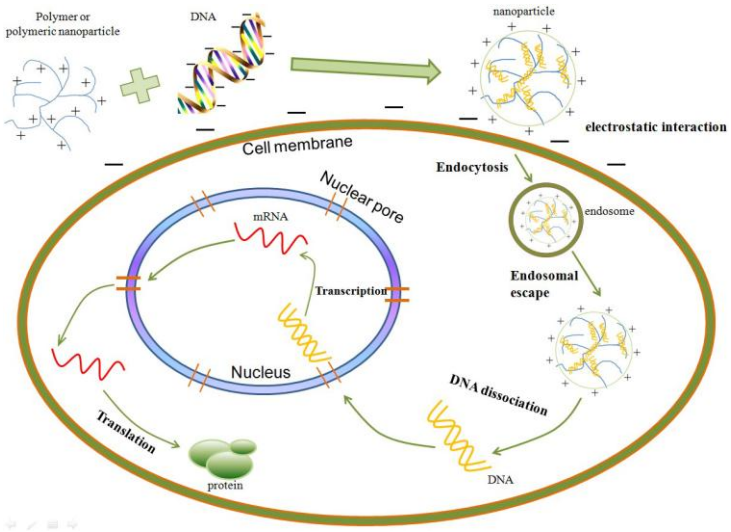


Figure 1.12: The schematic presentation of gene delivery process of polymers (Jin, Zeng, 2014).

1.4.1 Importance of charge inversion

The phenomenon of overcharge plays an important role in gene delivery. The Manning counterion condensation theory states that about 90% of the DNA charges need to be neutralized for the DNA to condense. The cell surface membrane of the living cells carries a large negative charge which is the same negative sign of DNA, that prevents the penetration of DNA as nacked-DNA through the cell membranes because of the electrostatic repulsion force between the DNA and cell membrane. A positively charge synthetic vector for DNA such as cationic lipids, cationic polymers can be used. The complexation between dendrimer and DNA happen if the DNA has to be overcharged with the oppositely charged dendrimer. Dendrimer carrying positive charge convert DNA charge to get positively net charge of complex, this makes complex overcome on negative charge of cell membrane to penetrate the cell membrane. This correlation theory, which has been studied by computer simulations.

1.4.2 DNA- Dendrimer wrapping process

Electrostatics interaction is the major leading force in the DNA- dendrimer wrapping process, when the dendrimer is positively charged due to the protonation of all the primary amines, the strong electrostatics interaction helps the DNA strand collapse onto the dendrimer (Maiti and Bagchi,2006). Transfection efficiency of DNA complexed with dendrimers depend on the size, structure and the charge density of these polymers. Condensation of DNA protects the DNA against degradation, transports DNA across membranes, by using dendrimer as DNA vector barrier towards gene delivery.

1.4.3 Effect of pH on PAMAM dendrimer complexation

It is as well of high importance to understand DNA compaction by dendrimers in terms of gene expression dependence on the condensed state (Fant., 2008). Dendrimer conformation adjusts to the solvent, pH and ionic strength. Molecular dynamics simulations predicted that PAMAM dendrimers structure has an extended conformation at low pH (pH<4) the interior becomes hollow with the increase in the generation number resulting from repulsions between primary and tertiary positively charged amines in the interior and surface of the dendrimer. A neutral pH decreases the size of the dendrimer, probably due to hydrogen bonding between uncharged tertiary amines and positively charged surface amines, the strong electrostatics interaction helps the DNA strand collapse onto the dendrimer. At high pH (pH>10) the dendrimer is uncharged and the formation of any DNA-dendrimer complex is not seen (Vedha Hari et al., 2012). Dendrimers contract since the global charge approaches neutrality, taking more spherical structure, where the repulsive forces between the surface groups and the dendrimer arms decrease (see Figure 1.13). In particular, DNA penetrates inside the dendrimers at neutral pH, while less penetration was reported at low pH, suggesting a better release from the complex when considering DNA delivery (Maiti and Bagchi, 2006). Principle parameters derived from the bead model are listed in Table 1.2.

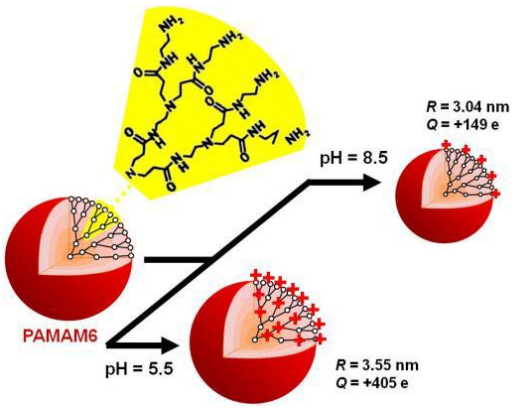


Figure 1.13: Schematic representation of PAMAM6 size and charge pH dependence (Dootz et al., 2011).

Table 1.2: Characteristic properties of PAMAM6 and PAMAM6/DNA derived from the bead model at pH 5.5 and 8.5(Dootz et al., 2011).

	pH = 5.5	pH = 8.5
PAMAM 6		
radius in solution R_{P6}	3.55 nm	3.04 nm
radius in complex R_{P6C}	3.3 nm	2.8 nm
total charge Q_{P6}	405 e^+	160 e^+
contribution of primary amino groups Q_I	256 e^+	160 e^+
contribution of tertiary amino groups Q_{III}	149 e^+	0 e^+
surface charge density Σ_{P6}	1.6-1.9 e^+/nm^2	1.46 e^+/nm^2
DNA		
absorbed amount of bp	135 bp	35 bp
absorbed length L_a	46 nm	12 nm
diameter d_{DNA}	2 nm	
line charge density τ_{DNA}	6 e^-/nm	
persistence length L_P[249]	50 nm	
covered dendrimer surface area A_{P6C}	92 nm^2	24 nm^2
absorbed charge Q_{DNA}	270 e^-	70 e^-
local overcharging (Q_{DNA}/A_{P6C}-Σ_{P6})	1.0-1.3 e^-/nm^2	1.46 e^-/nm^2
bending energy E_b	$62kT$	$22kT$

1.4.4 Dendrimers and the effect of salt:

Molecular simulations generally conclude that high ionic strength (high concentration of salts), has a strong effect on charged dendrimers and favors a contracted conformation of dendrimers, with a high degree of back-folding somewhat similar to what is observed upon increasing pH or poor solvation. At low salt conditions, the repulsive forces between the charged dendrimer fractions results in an extended conformation in order to minimize charge repulsion in the structure (see Figure 1.14).

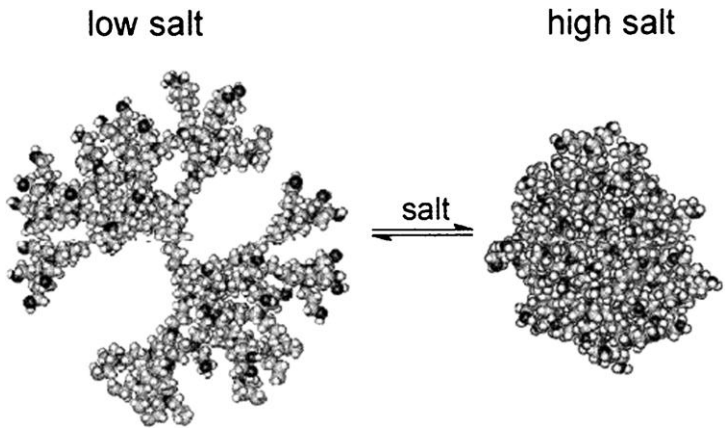


Figure 1.14: Show a three-dimensional conformational change of a PPI dendrimer upon increasing ionic strength (Boas et al., 2006).

1.5 Statement of the Problem

Through our study we like describe dendrimer as an ion penetrable sphere (soft sphere) as Qamhieh and Abu Khaleel did in a previous study, (Qamhieh and Abu Khaleel., 2011), where the ions can penetrate inside the dendrimer, (see Figure 1.15). They did their study for specific dendrimers, while we investigated the complexation between DNA and dendrimers of different generations (G1-G6) for Ammonia cored dendrimers and (G1-G10) for Ethylenediamine cored dendrimers. In our study we investigated the effect of some factors, like salt concentration, persistence length (l_p), dendrimer generation size, pH of the solution, and the strength of electrostatic interaction.

(a) (b)

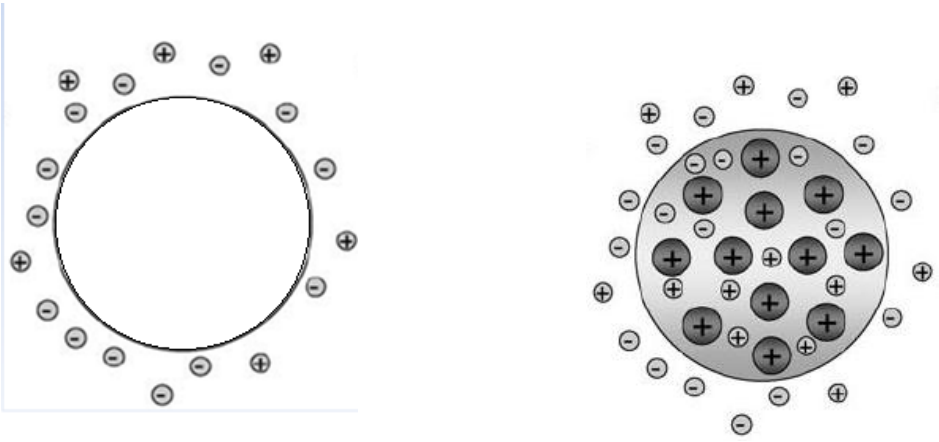


Figure 1.15: Graphic of (a) hard sphere model, and (b) a soft sphere (ion- penetrable) model.

Chapter Two

Model and Method

Chapter Two

2.1 Introduction

In this study we used a penetrable model for the complexation of DNA with dendrimer which has been developed by Qamhieh (Qamhieh et al., 2009), and used all the parameter into this model to find out its impact on the complex.

2.2 Analytical model of the system

Qamhieh and co-workers considered complexes formed between soft (ion penetrable) sphere of radius R and charge Ze, represent the dendrimer, and the DNA as a linear polyelectrolyte (LPE) chain of l_p persistence length, of radius r = 1 nm, length L>>R and the charge density of LPE is $\lambda = -e / b$, where b is the axial spacing between charges on the chain. The sphere and the LPE chain are placed in 1:1 salt solution in a system characterized by Bjerrum length $l_B = e^2/\varepsilon k_B T$, where ε is the dielectric constant of the medium, k_B is Boltzmann constant, T is the thermal energy, "T: absolute temperature, and a Debye screening length this $\kappa^{-1} = (8 c_s \pi l_B)^{-1/2}$ where c_s is the salt concentration.

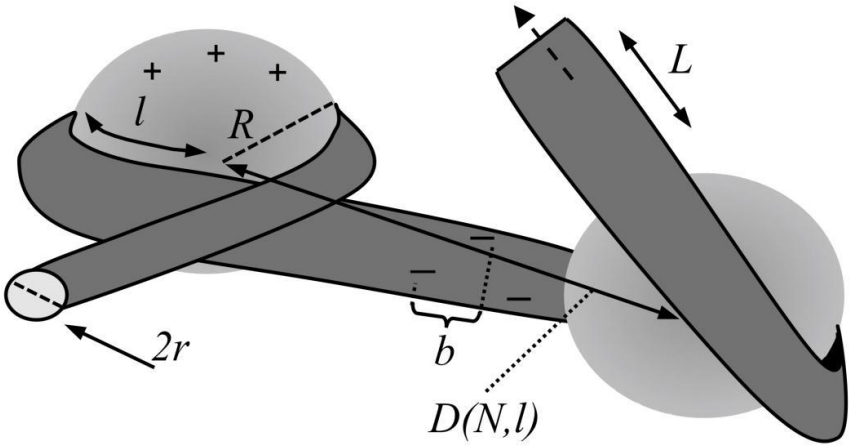


Figure 2.1: Proposed binding model between DNA of contour length *L*, radius *r*, and distance between negative charges *b* and G4 PAMAM dendrimers modeled as hard spheres of radius *R*. The DNA is shown to wrap around the dendrimer with the length of the wrapping part equal to *l*, and a distance between the centers of two neighboring dendrimers, *D* (*N*,*l*). The model is in accordance with the cooperative binding model proposed by Örberg on co-workers (Örberg et al., 2007).

2.3 The free energy for a single dendrimer/LPE complex

Bases on Schiessel's model (Schiessel et al., 2001) the total free energy of the system consisting of one dendrimer and one LPE chain is given as the following:

$$F(l) = F_{compl}(l) + F_{chain}(L-l) + F_{compl-chain}(l) + F_{elastic}(l) \qquad (2.1)$$

Where (*l)* is the length of the DNA molecule wrapped around the dendrimer sphere, and the remaining chain is of length (*L* _ *l*).

The first term, F_{compl} (l), is the electrostatic charging free energy of a spherical complex of charge $Z(l)e$

$$F_{compl}(l) \cong \frac{3Z^2(l)l_B k_B T}{8\pi(\kappa R)^2}\left[\cosh(\kappa R) - \frac{\sinh(\kappa R)}{\kappa R}\right]\frac{e^{-\kappa R}}{R} \qquad (2.2)$$

The complex, i.e., the sphere and corresponding wrapped chain has, a total charge $Z(l) = (Z\_ l/b)$.

From Schiessel's model the second term, F_{chain} $(L\_ l)$, the total entropic electrostatic free energy of the remaining chain $(L\text{-}l)$ given by:

$$F_{chain}(L-l) \cong \frac{k_B T}{b}\cdot\Omega(a)\cdot(L-l)\cdot(1-\xi^{-1}) \qquad (2.3)$$

Where $\Omega(a)$, is the entropic cost describing the condensed DNA counterion, $\xi = l_B / b$ is the so-called Manning parameter (Manning, 1978).

The third term, $F_{compl\text{-}chain}(l)$, is the electrostatic free energy of the interaction between the complex (ion penetrable sphere) and the rest of the chain, given by:

$$F_{compl-chain}(l) \cong \frac{3Z(l)l_B k_B T}{4\pi(\kappa R)^2}\left[\cosh(\kappa R) - \frac{\sinh(\kappa R)}{\kappa R}\right]$$
$$\times\left[\ln(r) - \sum_{n=0}^{\infty}\frac{(-1)^n}{(n+1)!\cdot(n+1)}\cdot(\kappa r)^{n+1}\right]\Bigg|_{R}^{L-l} \qquad (2.4)$$

The final term in Eq. (2.1), $F_{elastic}(l)$, is the elastic (bending) free energy required to bend (l) of the chain of radius of curvature around sphere of radius R is the same as the one used in Schiessel model

$$F_{elastic}(l) \cong \frac{k_B T l_p}{2R^2} l \qquad (2.5)$$

2.4 The free energy for the dendrimer/DNA aggregate

For a system consisting of one DNA molecule and N number of dendrimers, the total free energy can be expressed as:

$$F(l,N) = NF(l) + F_{\text{int}}(N,l) \qquad (2.6)$$

Where F(l) is the total free energy of the dendrimer–LPE chain complex, and F_{int} is obtained as the electrostatic repulsion interaction between the dendrimer spheres, which are decorating the DNA molecule.

For the case of the interaction between complexes each of charge $Z(l)e$, the interaction free energy can be obtained by summing over the electrostatic repulsion between all complexes within one chain.

$$F_{\text{int}}(N,l) = \frac{9Z^2(l)k_BTl_B}{8\pi(\kappa R)^4}\left[\cosh(\kappa R) - \frac{\sinh(\kappa R)}{\kappa R}\right]^2$$
$$\times \sum_{i=1}^{N-1}\left[\left[\frac{N-i}{i}\right]\frac{e^{-\kappa D(N,l)}}{D(N,l)}\right] \qquad (2.7)$$

Where $D(N,l)$ the center-to-center distance between two neighboring complexes which equals to $(L-Nl+2NR)/N$, is small compares to (κ^{-1})very low ionic strength but larger than 2R (no excluded volume effects),This interaction energy worths nothing that this term will be small if the charge of complex is close to neutral.

Finally the total free energy of the system is:

$$\frac{F(N,l)}{k_BT} = \frac{3NZ(l)l_BA}{4\pi(\kappa R)^2}\left[\frac{Z(l)}{2}\frac{e^{-\kappa R}}{R} + \frac{1}{b}\cdot\int_R^{L-Nl}\frac{e^{-\kappa r}}{r}dr + \frac{3Z(l)A}{2(\kappa R)^2}\sum_{i=1}^{N-1}\left(\frac{N-i}{i}\right)\frac{e^{-\kappa D}}{D}\right]$$
$$+\frac{1}{b}\cdot\Omega(a)\cdot(1-\xi^{-1})\cdot(L-Nl) + \frac{Nl_p}{2R^2}l. \qquad (2.8)$$

Chapter Three

Results and Discussions

Chapter Three

Results and Discussions

For a system of LPE chain and PAMAM dendrimer of different generations, the optimal wrapping length (l_{opt}) of LPE chain which has been wrapped around dendrimer can be found by taking the first derivative of total free energy equation (Eq. 2.8) for the penetrable sphere model.

3.1 Single PAMAM dendrimer- LPE chain complex

The optimal wrapping length (l_{opt}) of LPE chain, which has been wrapped around dendrimer, can be solved by taking the first derivative of the free energy equation (Eq.(2.8)) for soft sphere model, with respect to the wrapping length (l) and equal it to zero.

The complexation of LPE chain and single penetrable sphere, representing dendrimer, in a 1:1 salt solution of NaBr has been studied by the penetrable sphere model with divalent (represents dsDNA) LPE chains, the charge spacing b= 0.17nm has been considered. Effect of different factors on the complexation at constant length of DNA; 2000 basepair (bp; L= 680 nm) is studied, such as dendrimer size generation, the Bjerrum length, salt concentration, pH of the solution, and rigidity of the LPE chain (Persistence length).

3.1.1 Effect of dendrimer size generation on optimal length condensed on PAMAM dendrimer:

To cover the effect of different generation on the complex formation, we took Ammonia core PAMAM dendrimer of different generation (G1, G2, G3, G4 and G6) and ethylenediamine cored PAMAM dendrimer of different generation (G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10) to compare the change in behavior of each one when the charge and radius change for each generation of dendrimer, at constant parameter of the complexation. The system is composed of the flexible LPE chain of persistence length l_p= 3nm, charge spacing of the chain is b= 0.17nm, and length L= 680 nm, and the penetrable sphere model at 1:1 salt concentration corresponding to Debye screening length (DSL) of 6nm, have been studied at different generation dendrimer using penetrable sphere model with PAMAM dendrimer.

Figure 3.1 shows that the optimal DNA wrapping length around the dendrimer has increased for higher dendrimer generation, that the number of DNA turns around a dendrimer increases with the dendrimer size or generation. This finding is in agreement with experimental studies by Ainalem et al. and Carnerup et al. reporting on dendrimer/DNA aggregate morphologies. It should also mention that the shape of the dendrimers changes with generation, higher generation are typically more spherical than the lower generation that has a more disc-like shape (Lee et al.,2002, .Paulo et al., 2007,).

The highly ordered rods and toroids found for low generation dendrimers, can be attributed to the fact that the DNA wraps less than one turn around the dendrimer. Consequently the disordered globular structures that appear for high generation

dendrimers, is likely a consequence of the wrapping of DNA in several turns around these dendrimers (see Table 3.1).

Figure 3.2 supports the result of ammonia cored to explain that the optimal wrapping length increases as the generation of dendrimer increases with linear relation. This large growth attributes the fact that the number of functional primary amine groups increases exponentially with generation. The higher generation dendrimers, have both lower curvature and higher surface charge density. They are therefore able to interact more effectively with the DNA charges at the same time as the free energy cost of bending the DNA is less than for lower generation. In fact low generation (G1-G5) can be regarded as decorating the DNA chain. For higher generation (G6-G10) toroidal aggregates form, the electrostatic attraction is expected to be moderate as that balance between mobility and high affinity binding of the DNA to the dendrimer exists (see Table 3.2).

The complex of DNA and a penetrable single sphere of radius R = 3nm has been studied. According to previous theoretical studies that have used a sphere of different charges Z = 10, 20, and 100, and 2000 bp DNA (L=680) with persistence length (l_p=3nm), at100mM salt solution and 0.71nm of Bjerrum length. Table 3.3 shows overcharging degree of sphere calculated in terms of $(l_{opt} - l_{iso})/l_{iso}$, where l_{iso} is the isoelectric length of the chain. Our results are summarized in Table 3.3with a previous results predicted by a hard sphere model. The overcharging degree in the case of penetrable sphere shows larger values compared with hard sphere models. This explains the difference in values of the total electrostatic interaction free energies of single complex for both penetrable sphere and hard sphere models. We found that the overcharging degrees are 12.28, 5.91, and 0.82 for charges number 10, 20, and 100 respectively, to be compared with results obtained

previously for the hard sphere models by Netz and co-workers (Kunze and Netz, 2002) and Arcesi and co-workers (Arcesi, et al., 2007).

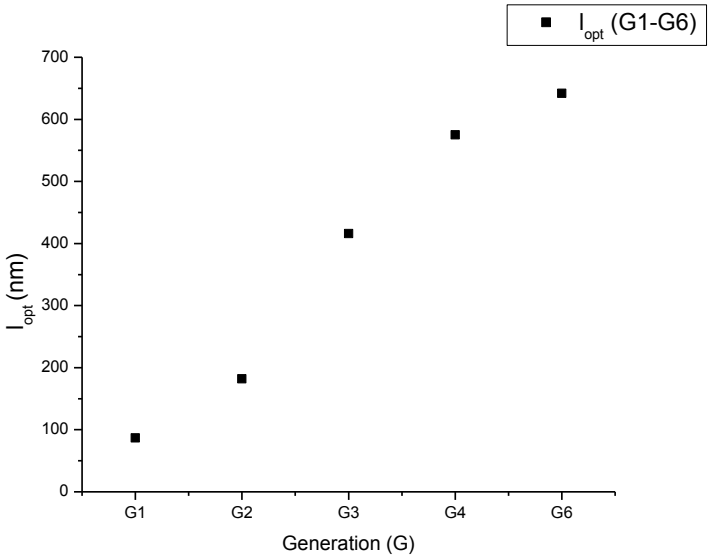


Figure 3.1: Effect of different generation of ammonia cored dendrimer on optimal wrapping length around LPE chain of persistence length l_p = 3nm, charge spacing of the chain is b= 0.17nm, and length L= 680 nm, and the penetrable sphere model at 1:1 salt concentration corresponding to Debye screening length (DSL) of 6nm.

Table 3.1: The overcharging degree of ammonia cored sphere of different size generation by DNA of 2000bp (L=680nm).

Generation	l_{opt}	$l_{opt}/2\pi R$	Z^*_{compl}	Overcharging degree $(l_{opt} - l_{iso}) / l_{iso}$
G1	86.6	8.7	-503.4	83.9
G2	182	13.1	-1058.5	88.2
G3	416	20.7	-2423.0	100.9
G4	575	22.89	-3334.3	69.4
G6	650	34.5	-3631.5	18.91

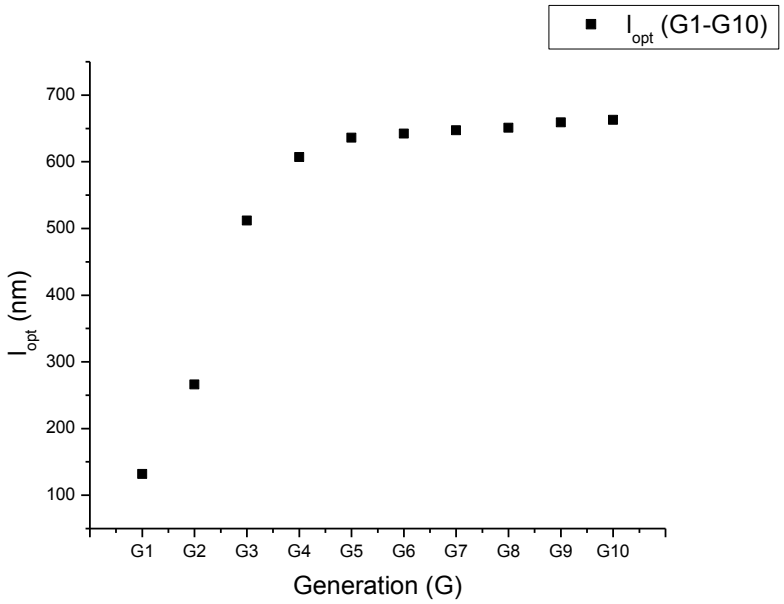


Figure 3.2 : Effect of dendrimer size generation of ethylenediamine cored dendrimer on optimal wrapping length around LPE chain of persistence length l_p = 3nm, charge spacing of the chain is b= 0.17nm, and length L= 680 nm, and the penetrable sphere model at 1:1 salt concentration corresponding to Debye screening length (DSL) of 6nm.

Table 3.2: The overcharging degree of ethylenediamine cored sphere of different size generation by DNA of 2000bp (L=680nm).

Generation	l_{opt}	$l_{opt}/2\pi R$	Z^*_{compl}	Overcharging degree $(l_{opt} - l_{iso}) / l_{iso}$
G1	132	1.10	-768.4	96.0
G2	266	16.29	-1548.7	96.7
G3	512	22.64	-2979.7	93.1
G4	607	21.96	-3506.5	54.7
G5	636	17.76	-3613.1	28.2
G6	642	14.19	-3520.4	13.7
G7	647	12.7	-3293.8	6.43
G8	651	10.68	-2805.4	2.73
G9	659	9.20	-1828.4	0.89
G10	663	7.82	196	-0.9

Table 3.3: The overcharging degree of sphere of R = 3nm by DNA of 2000bp (L=680nm) at concentration of 1:1 salt solution.

			Overcharging degree $(l_{opt} - l_{iso}) / l_{iso}$			
Charge number Z	l_{opt}	No. of Turns	Penetrable sphere model	Penetrable sphere model (Qamhieh, K., Abu Khaleel, A., (2011))	Hard sphere model (Arcesi et al., 2007)	Hard sphere model (Kunze and Netz, 2002)
10	360	19.1	210.7	12.28	7.05	6.23
20	363	19.2	105.7	5.91	3.52	3.11
100	375	19.9	21.0	0.84	0.71	0.62

3.1.2 Effect of Bjerrum length on optimal chain length condensed on PAMAM dendrimer:

The interaction between positively charged poly amidoamine (PAMAM) dendrimers of different generation, in the protonated state, with LPE chain has been investigated. The dendrimer can be modeled as a penetrable sphere of different charges, such a charge pattern corresponds to physiological (neutral) pH conditions (Koper, 1998). The systems have been studied by the flexible LPE chain of persistence length l_p= 3nm, and length L= 680 nm, and the penetrable sphere model at 1:1 salt concentration corresponding to Debye screening length (DSL) of 6nm. Optimal wrapping length of the LPE chain of charge –e on the PAMAM dendrimer of different generation has been estimated using the penetrable sphere at different values of Bjerrum length, which is a measure of the strength of electrostatic interactions between LPE chain and dendrimer complexes. Therefore when Bjerrum length increases i.e. the strength of electrostatic interactions increases, this leads to a major reduction in the positive charge of the dendrimer/chain complex and reduces its potential ability to bind to the plasma membrane.

Figure 3.3 shows that results of the fraction of the optimal length of condensed monomers of the LPE chain on the dendrimer (l_{opt}) increases as Bjerrum length increases, this has been calculated by the penetrable sphere model. Small values of Bjerrum length means the electrostatic interactions are weak, l_B< b, there is small fraction of the condensed divalent chain on dendrimer, that a substantial chain tails have not been condensed on the dendrimer. These tails are getting shorter very quickly as the Bjerrum length is increased, and are about to disappear for l_B≈ b, where the divalent LPE chains

are achieved saturation and completely condensed on the PAMAM dendrimer for different generation. In divalent DNA strands complex, we noted that high generation (G6 and G4) is effected by Bjerrum length change, more than low generation (G1, G2, and G3) due to the difference in charge value. But we also mentioned that low generation ammonia cored is less effected in the change in Bjerrum length than high generation ammonia cored due to the difference in charge and radius value which influence the electrostatic interaction.

Figure 3.4 shows the effect of Bjerrum length on the complexation of ethylenediamine cored PAMAM dendrimer of different generations (G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10) with same flexible LPE chain of length L= 680 nm, persistence length l_p= 3nm and Debye screening length = 6nm, so we note that the small fraction of the condensed monomers on dendrimer is a weak electrostatic interaction, l_B< b, which means that the tail is the dominant and this tail are getting shorter rapidly as Bjerrum length is increased, and for $l_B \approx$ b disappears. The figure also indicates that the effect of Bjerrum length is different from one generation to another of the same cored according to the difference in charge and radius value which causes difference in charge distribution on the dendrimer.

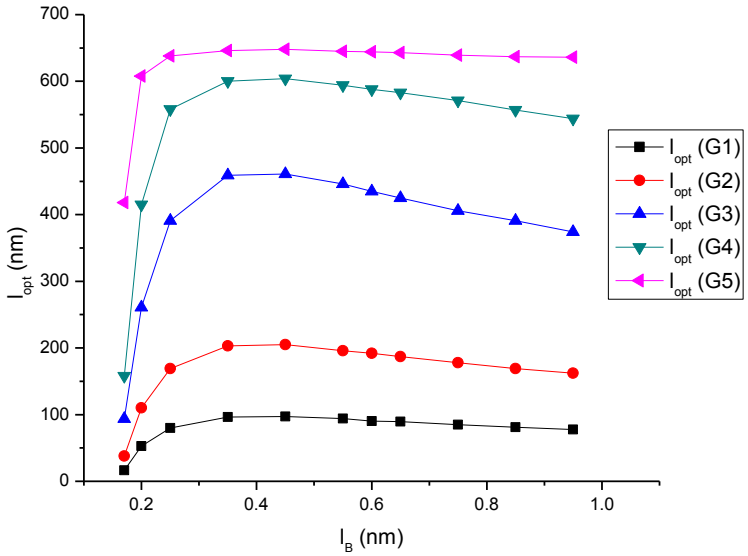


Figure 3.3: Optimal length condensed on a charged dendrimer of different generations of ammonia cored with divalent LPE chain as a function of Bjerrum length l_B. The dendrimer is complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

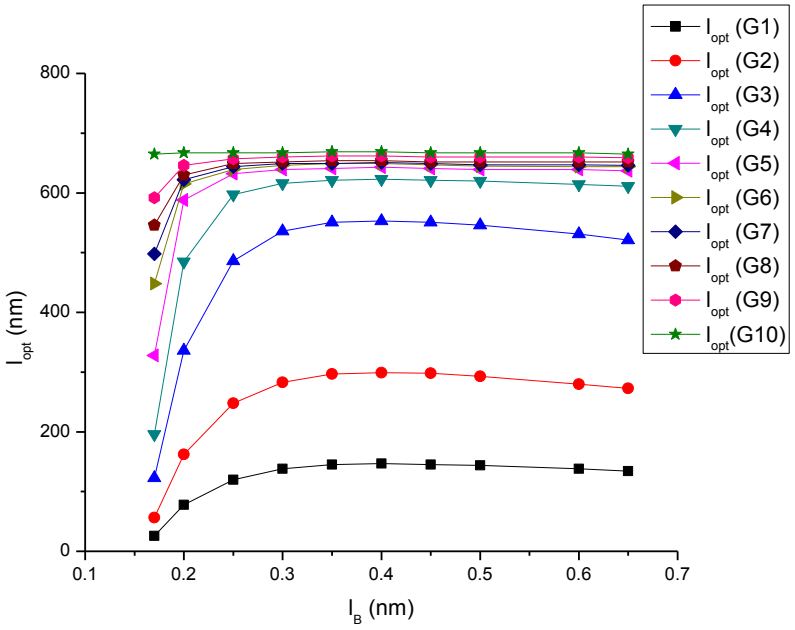


Figure 3.4: Optimal LPE chain condensed on a charged dendrimer of different generations of ethylenediamine has cored with divalent LPE chain as a function of Bjerrum length l_B. The dendrimer is complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

Figure 3.5 shows a comparison between complexation of ammonia and ethylenediamine cored PAMAM dendrimer of different generations with DNA and concludes that the optimal length condensed of the LPE chain on the dendrimer of both types (l_{opt}) is increasing as Bjerrum length increases, but the optimal length condensed on ethylenediamine cord dendrimer is larger than it is on ammonia cored dendrimer of the same generation. We noted from figure that G4 and G3 for both types are effected by Bjerrum length change more than G2 and G1, due to the difference in charge and value which influence the electrostatic interaction.

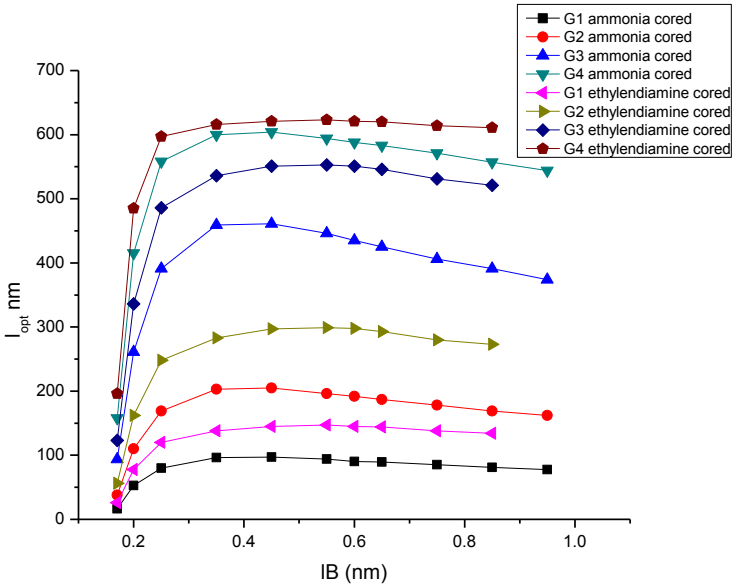


Figure 3.5: Optimal length condensed on a charged dendrimer of different generations of ammonia and ethylenediamine cored with divalent LPE chain, as a function of Bjerrum length l_B. The dendrimer is complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

3.1.3 Effect of salt concentration on optimal chain length condensed on PAMAM dendrimer:

The complexation of flexible LPE of 680 nm length chain and persistence length l_p=3 nm with an oppositely charged Ammonia core PAMAM dendrimer of different generations (G1, G2, G3, G4, G5 and G6) and ethylenediamine cored of (G1, G2, G3, G4,G5, G6, G7,G8, G9, G10) have been studied at different concentrations of 1:1 salt solution at room temperature which gives 0.71 nm of Bjerrum length. The LPE chain modeled DNA molecule of 2000 bp and charge spacing of the chain is b= 0.17nm. Table 3.4 shows the optimal wrapping length of LPE chain around ammonia cored dendrimers of different generations G1, G2, G3, G4, G5 and G6. In Figure 3.6 Shows the calculated fraction of optimal length condensed on the ammonia cored dendrimers of different generations as a function of reciprocal Debye length κ, which is proportional to salt concentration, so we can conclude that when the salt concentration increases at small values of κ the optimal length increases, at higher salt concentration the optimal length is almost fixed due to a finite length of LPE chain. The effect of salt concentration on the optimal wrapping length depends on the strength of the electrostatic interaction that is measured by Bjerrum length l_B. For the total free energy (Eq.(2.8)) that tends the LPE chain to resist the wrapping around dendrimer namely, the mechanical bending free energy and the electrostatic repulsive free energy between the chain monomers, the latter loses its importance with the increase of salt concentration, these repulsions are balanced by the electrostatic attraction between dendrimer – LPE chain complex, which favors the bending of LPE chain in order to wrap around dendrimer (Kunze and Netz, 2002). At higher salt concentration it is observed that the overcharging degree of dendrimer

becomes larger. Also it is shown from the figure that the optimal wrapping length around dendrimers of generations G1 and G2 has increased by the increase of salt concentration. With G3 dendrimer and at low salt concentration the optimal length increases by increasing the salt, at high salt concentration the effect is insignificant. With G4,G5,and G6 at low salt concentration the optimal wrapping length is larger, and decreased by a very small value as the salt concentration is increased, which means that the effect of salt concentration is almost insignificant on the complexation of LPE chain G4,G5 and G6 dendrimers.

In Figure 3.7 we study the salt concentration effect on ethylenediamine cored PAMAM dendrimer of (G1-G10).Lower generation (G1and G2), the fraction of the condensed monomers on dendrimer increases by increasing salt concentration (see Table 3.5). With dendrimers at low salt concentration, the optimal length increases by increasing the salt, at high salt concentration the effect of salt concentration is insignificant. For high generation (G9 and G10) the size of the condensed DNA aggregates does not change, which means that the aggregate formed from the complexation between LPE chain and dendrimer of higher generations seemed to be more neutralized, because more and more of DNA charges get more neutralized by higher generations, this trend is found in agreement with the recent experimental study (Carnerup, et al., 2011).

Table 3.4: Analytical model results from the interaction between ammonia cored dendrimer of (G1-G6) and the longer DNA (2000bp), the dendrimer is considered to be a penetrable sphere of radius *R*.

Cs (mM)	l_{opt}(nm)					
	G1	G2	G3	G4	G5	G6
10	96.8	221	512	613	630	632
30	123	303	584	612	614	605
50	142	363	595	609	602	590
100	197	488	593	590	577	568
120	217	514	589	586	572	566
150	249	536	585	579	568	564
200	297	556	578	572	563	564

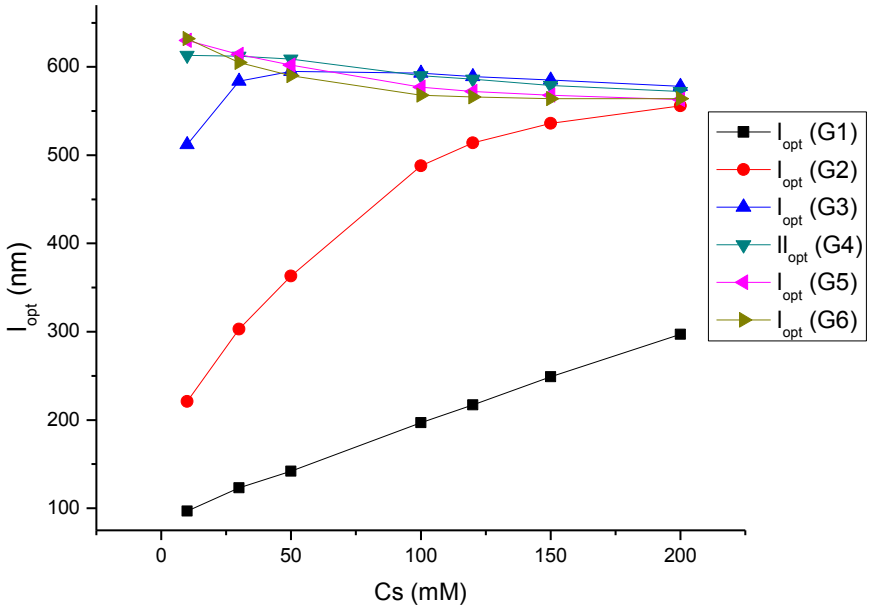


Figure 3.6 : Optimal length of LPE chain on ammonia cored PAMAM Dendrimer Gx as a function of concentration of 1:1 salt solution, a constant length of flexible LPE equals to L = 680nm with spacer of b=0.17nm, of persistence length equals to 3nm.

Table 3.5: Analytical model results from the interaction between ethylenediamine cored dendrimer of (G1-G10) and the longer DNA (2000bp), the dendrimer is considered to be a penetrable sphere of radius *R*.

Cs (Mm)	l_{opt} (nm)									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
10	154	335	581	621	633	633	633	633	642	667
30	203	459	605	616	613	602	599	600	617	666
50	243	519	604	609	598	588	584	589	612	666
100	345	571	594	589	573	570	571	584	612	664
120	384	575	588	580	570	567	571	584	612	664
150	426	578	582	575	567	565	571	584	612	664
200	481	579	576	568	563	563	571	584	612	662

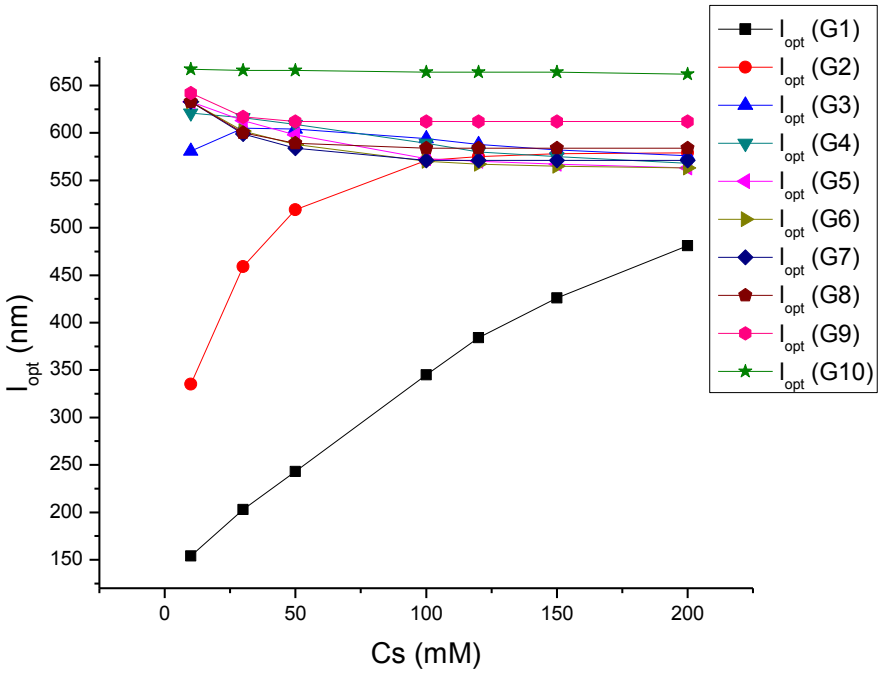


Figure 3.7 : Optimal length of LPE chain as a function of 1:1 salt concentration for a system of one positively charged on ethylenediamine cored PAMAM dendrimer of Gx and an appositively charged LPE chain of persistence length l_p= 3nm representing DNA of 2000bp (L=680 nm), the dendrimer is considered to be penetrable sphere of radius R.

In addition we study the comparison of salt concentration effect on ethylenediamine and ammonia cored PAMAM dendrimer, and have the same result, when the salt concentration increase at small values of κ the optimal length increase, and this depends on the strength of the electrostatic interaction that is measured by Bjerrum length l_B (see Figure 3.8).

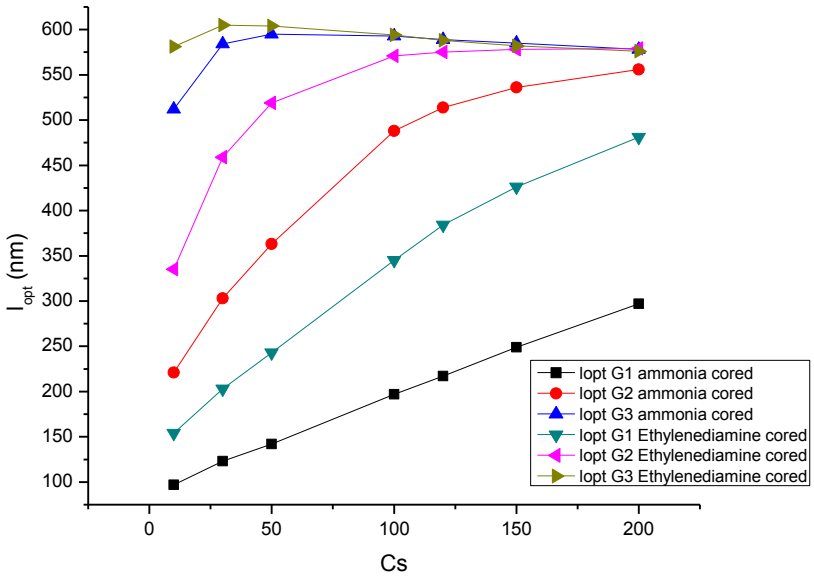


Figure 3.8: Optimal length of LPE chain as a function of 1:1 salt concentration for a system of positively charged on ammonia and ethylenediamine cored PAMAM dendrimer of Gx and an appositively charged LPE chain of persistence length l_p= 3nm representing DNA of 2000bp (L=680 nm), the dendrimer is considered to be penetrable sphere of radius R.

3.1.4 Effect of Persistence length on optimal chain length condensed on PAMAM dendrimer:

The complexation of flexible LPE chain of length L=680nm (2000 bp), with oppositely charged of ammonia cored and ethylenediamine cored at different generation have been studied, using the penetrable model, at different degrees of chain rigidity measured in terms of persistence length (l_p). The values of Bjerrum length are considered, l_B = 0.71nm represents the complex in water solution. The complexation has been studied with divalent (represents dsDNA) LPE chains, with charge spacing b=0.17nm has been used.

Figure 3.9 shows the optimal wrapping length l_{opt} as a function of persistence length in all cases with oppositely charged of ammonia cored sphere at different generations (G1, G2, G3, G4 and G6). From the figure, we can accord the decrease of optimal wrapping length around dendrimer, in all cases as the rigidity of the chain is increased, this effect of Bjerrum length is great in water solution for double chain (b=0.17nm). The decrease in optimal length rounds dendrimers is more rapid and more considerable with small generations (G1,G2, G3 and G4) with radius R= 1.58, 2.2, 3.2 and 4nm respectively, with divalent chain, the decrease is most rapid and the length of wrapping length is the largest.

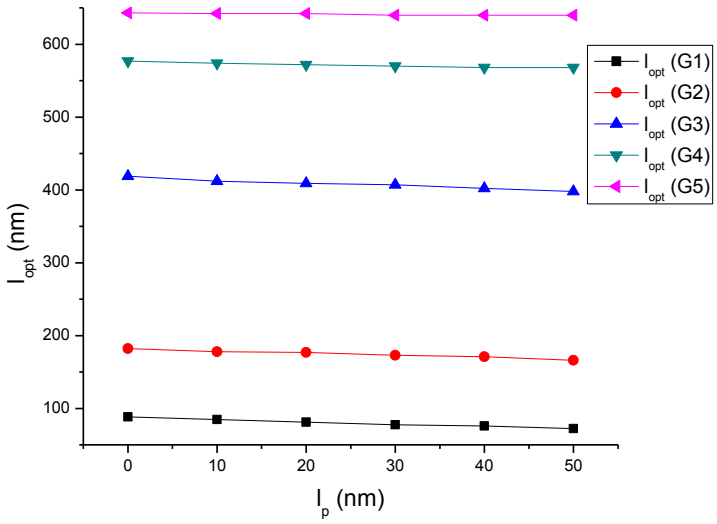


Figure 3.9: Optimal chain length l_p condensed on ammonia cored dendrimers at different generations as a function of persistence length, for a system an oppositely charged LPE of length L=680nm, at 1:1 salt concentration.

Figure 3.10 shows that the decrease in optimal length round the dendrimer, is more rapid and significant with small radius of ethylenediamine cored PAMAM dendrimers generations G1, G2,G3 and G4, at high generations (G5, G6, G7, G8, G9 and G10) the decreased in optimal length became slightly close to be fixed. Therefore we have concluded that the wrapping length is decreasing even more, when the radius of dendrimer is decreasing from high radius to low radius of sphere, as the bending energy is increased by increasing the radius of the sphere.

Figure 3.11 shows that the effect of chain stiffness is different from ammonia cored to ethylenediamine cored dendrimers according to the difference in radius, charge value and generation size of dendrimer. So when the rigidity of chain is increased, the optimal wrapping length around dendrimer decreased, this is clearer in low generation G2.

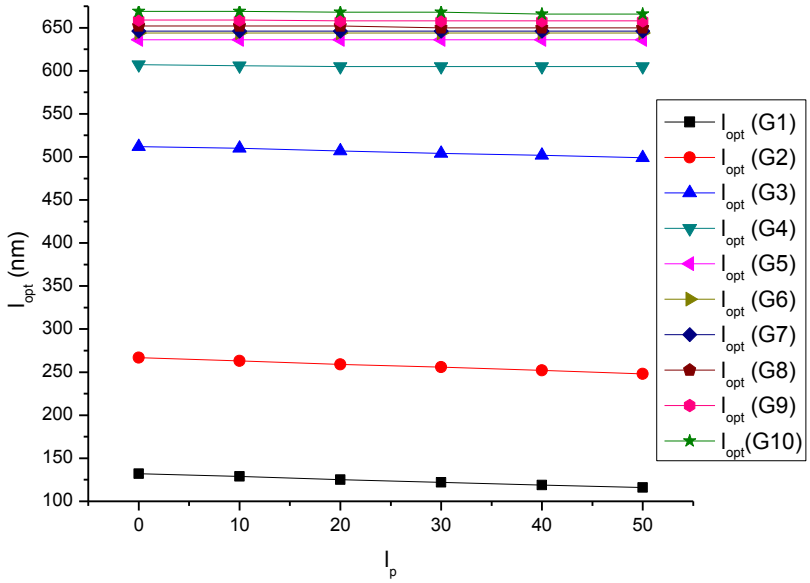


Figure 3.10: Optimal chain length l_p condensed on ethylenediamine cored dendrimers at different generations as a function of persistence length, for a system an oppositely charged LPE of length L=680nm, at 1:1 salt concentration.

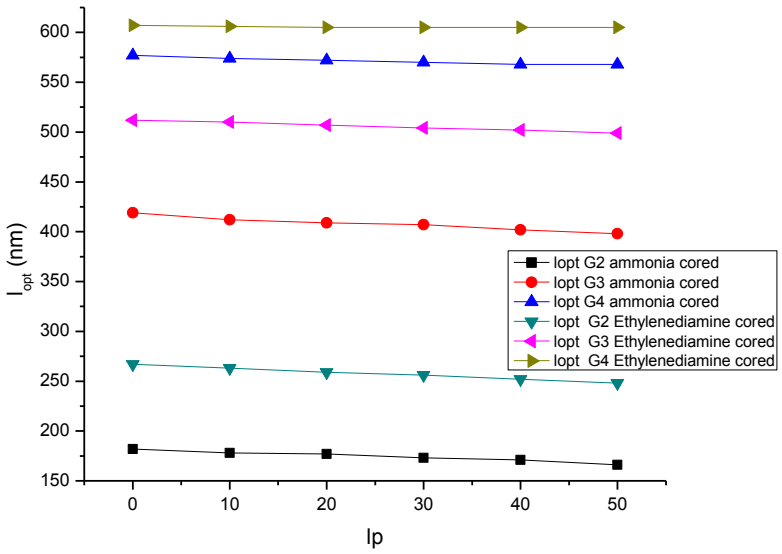


Figure 3.11: Optimal chain length l_p condensed on ammonia and ethylenediamine cored dendrimers at different generations as a function of persistence length, for a system an oppositely charged LPE of length L=680nm, at 1:1 salt concentration.

3.1.5 Effect of pH on optimal chain length condensed on PAMAM dendrimer:

At high pH the dendrimer is uncharged and DNA-dendrimer complex is not a formation and all amino groups of PAMAM dendrimers are deprotonated. At neutral pH, the dendrimer is positively charged due to the protonation of all the primary amines and hydrogen bonding between uncharged tertiary amines and the strong electrostatics interaction helps the DNA strand collapse onto the dendrimer. At low pH extended conformation of dendrimer exist due to intramolecular attraction happened between primary and tertiary amine are protonated, this reduces folding of dendrimer branch (see Table 3.6, 3.7). The composition formed between flexible LPE of 680 nm length chain (2000 bp) and persistence length l_p=3 nm with charge spacing of the chain is b= 0.17nm, and two PAMAM dendrimer of G2 of both ammonia and ethylenediamine cored have been studied, by applying the penetrable sphere model, in aqueous solutions containing 10mM 1:1salt which corresponds to 6nm of Debye screening length and 0.71nm of Bjerrum length.

Figure3.12shows the optimal wrapping length l_{opt} of ammonia cored and ethylenediamine sphere at generation (G2),by using a fix total charge degree are 405e and 160e and radius 3.55nm and 3.04nm for each pH value 5.5 and 8.5 respectively, and different values of total charge and radius according to all generations in neutral pH (pH=7) we can accord the increase of optimal wrapping length around dendrimer in acidic condition, and small wrapping length high pH value.

Table 3.6: Analytical model results from the interaction between ammonia core G2 dendrimer and DNA.

pH value	Radius (nm)	Total charge	l_{opt} G2(nm)
5.5	3.55	405	542
7	2.2	2	180
8.5	3.04	160	396

Table 3.7: Analytical model results from the interaction between ethylenediamene core G2 dendrimer and DNA.

pH value	Radius (nm)	Total charge	l_{opt} G2(nm)
5.5	3.55	405	542
7	2.6	16	266
8.5	3.04	160	396

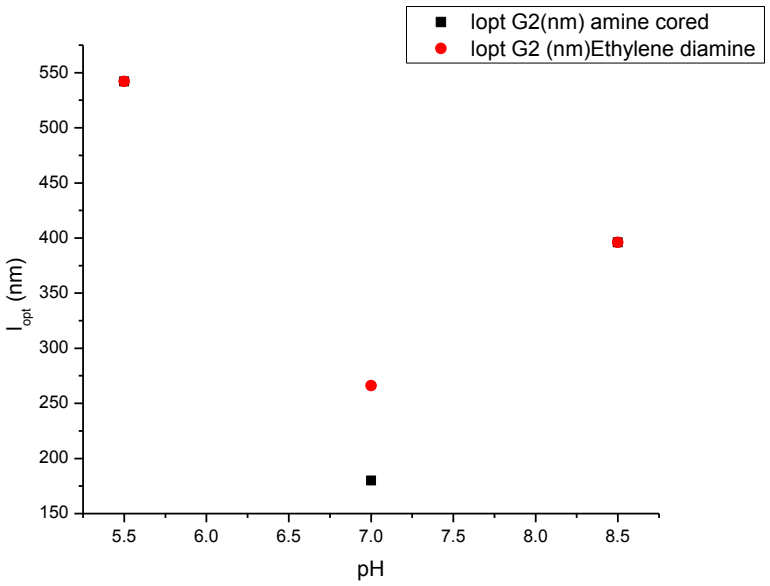


Figure 3.12: Optimal chain length condensed on ammonia and ethylenediamine cored denndrimers at G2 as a function of pH value, for a system of an oppositely charged LPE of length L=680nm, at 1:1 salt concentration.

3.2 System of a multiple PAMAM dendrimers-LPE chain complexes

3.2.1 Effect of PAMAM dendrimer contraction on the structural properties of dendrimers – LPE aggregate:

Dendrimers are known to be soft structure of high flexibility (Rosenfeldt et al., 2002; Likos et al., 2002). When dendrimers interact with an oppositely charged DNA, the internal structure and size of dendrimer are controlled, this is due to electrostatic repulsion between them. This shows to the reduction of the size of dendrimer as the radius changes from R=xR_0 will be taken into account in accordance to Qamhieh and co-workers study (Qamhieh et al., 2009) to exceed the experimental observations (Dootz et al., 2011).

Systems are composed of aggregates, formed between the flexible LPE chain (2000 base bair (bp)) and two PAMAM dendrimers of G4 of ammonia and ethylenediamine cored have been studied with persistence length l_p = 3nm, charge spacing of the chain is b= 0.17nm, and length L= 680 nm, and the penetrable sphere model at 1:1 salt concentration corresponding to Debye screening length (DSL) of 6nm.

Tables 3.8and 3.9 show the optimal wrapping length of DNA around ammonia and ethylenediamine cored PAMAM dendrimer of G4 reduce when dendrimer radius is decreased. As a result, the difference between the optimal wrapping length and the isoelectric length ($Diff = l_{opt} - l_{iso}$) is decreased. The net charge of the nucleosome of G4 Dendrimer – DNA complex remains decreased and always negative when dendrimer shrink upon the interaction with an oppositely charged LPE chain. This means that the

charge of dendrimers is inverted; the absolute value of the charge inversion ratio of the G4 – DNA nucleosome $|Z_{compl}(l)/Z_{dend}|\times 100\%$. Also the center-to-center spacing between complexes *D(N,l)* was shown to decrease when *R* reduced. This explains the increasing in the ratio of the electrostatic repulsion free energy, between complexes to the total minimized free energy of aggregate. Nevertheless, the linker between complexes ($D' = D - 2R$)isn't affected by the reduction of the size of the dendrimer. It has a constant value which means that the surface contact between two complexes, are kept at fixed separation, irrespective how much the dendrimer contracts. The ratio between the optimal wrapping length (l_{opt}) and the circumference of complex was found to increase when dendrimer *R* is decreased. The ratio between the un-neutralized length of DNA and the contour length of DNA is not constant, which gives the neutralized part of the DNA.

Table 3.8: Analytical model results from the interaction between G4 ammonia cored dendrimers and the DNA (2000bps), $N = 35$ and $L = 680$ nm, $Z_{dend} = 48$ and L_{iso} is found 8.16.

x (R=xR_o)	l_{opt} (nm)	*Diff* (nm)	Z^*_{compl}	Z^*_{compl}/Z	$D(N,l)$ (nm)	$D'(N,l)$ (nm)	$(D'+diff)N/L$	*Ratio* $l_{opt}/2\pi R$
1.00	15.8	7.64	-44.9	-0.93	3.15	-4.85	0.14	0.62
0.90	15.5	7.34	-43.17	-0.89	2.35	-4.85	0. 12	0.68
0.80	15.1	6.94	-40.82	-0.85	1.55	-4.85	0.10	0.75
0.70	14.7	6.54	-38.47	-0.80	0.75	-4.85	0.08	0.83
0.60	14.2	6.04	-35.52	-0.74	-0.04	-4.85	0.06	0.94
0.50	13.8	5.64	-33.17	-0.69	-0.84	-4.85	0.04	1.09
0.40	13.4	5.24	-30.82	-0.64	-1.64	-4.85	0.02	1.33

Table 3.9: Analytical model results from the interaction between G4 ethylenediamine cored dendrimers and the DNA (2000bps), N = 35 and L = 680 nm, Z_{dend} = 64 and l_{iso} is found 10.88.

x (R=xR_o)	l_{opt} (nm)	*Diff* (nm)	Z^*_{compl}	Z^*_{compl}/Z	$D(N,l)$ (nm)	$D´(N,l)$ (nm)	$(D'+diff)N/L$	*Ratio* $l_{opt}/2\pi R$
1.00	15.4	4.52	-26.5	-0.41	-0.34	-4.85	-0.01	1.08
0.90	15.2	4.32	-25.4	-0.39	-0.80	-4.85	-0.02	1.19
0.80	14.9	4.02	-23.6	-0.36	-1.24	-4.85	-0.04	1.31
0.70	14.7	3.82	-22.4	-0.35	-1.70	-4.85	-0.05	1.49
0.60	14.5	3.62	-21.2	-0.33	-2.14	-4.85	-0.06	1.71
0.50	14.2	3.32	-19.5	-0.30	-2.60	-4.85	-0.07	2.01
0.40	14	3.12	-18.3	-0.28	-3.04	-4.85	-0.08	2.47

3.2.2 Linker formation between complexes:

In this part we study the effect of LPE chain length, Bjerrum length and pH value on the linker formed between 2Gx-LPE chain and soft sphere complexes.

Figure 3.13a shows the effect of LPE chain length on linker formation of G3 dendrimers for ammonia core. The total length of LPE chain is divided into two parts, the linker and the optimal wrapping length, so we can estimate that the optimal wrapping length increases with increase of LPE length and linker. Comparing with the result by BD simulations (Larin et al., 2010), figure 3.13b shows smaller linker formation, this linker is shown to increase slowly with the increasing of the chain length.

Figure 3.14 shows the effect of Bjerrum length on the linker formed between 2G3 dendrimers for ammonia core with LPE chain of length 80nm. As it is shown when the optimal wrapping length increases, the ratio of l_B /b increases as a result, the linker decreases. This signify the increasing of the number of the chain condensation on the dendrimers, caused by reduction of the linker between complexes, in addition to the reduction of positive charge of the complex, as a result, the repulsive electrostatic interaction between complexes decreases.

We display in Figure 3.15 the effect of pH values on the linker formed between 2G6-LPE chain complex which gives Bjerrum length l_B=0.71and 100nm of Debye screening length, the LPE chain has persistence length of 3nm and spacer of 0.7nm. We note in acidic conditions, optimal wrapping length of LPE chain around dendrimer increase and the linker decrease.

(a) (b)

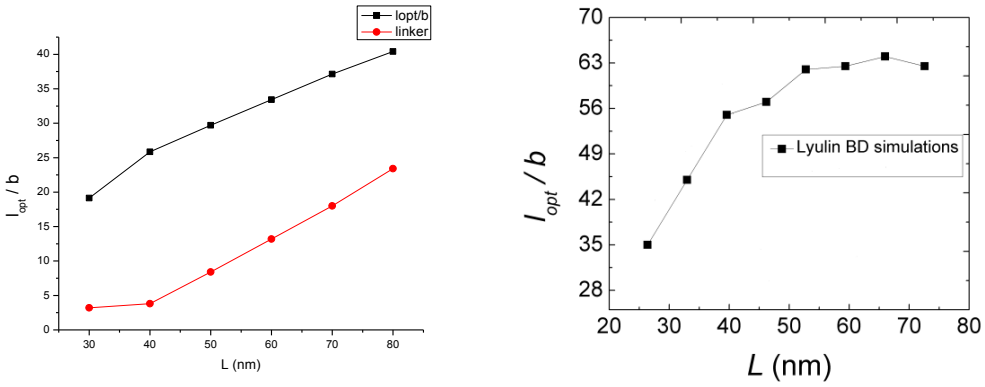


Figure 3.13: Effect of LPE chain length on optimal wrapping length and linker formation,(a) for complexes of 2G3 dendrimer and flexible LPE chain of spacer of 0.7nm and persistence length 3nm, 100nm of Debye screening length,(b), (Larin et al., 2010).

Table 3.10: Analytical model results from the interaction between 2G3 dendrimers and LPE chain of different LPE length.

L (nm)	l_{opt}/b (nm)	Linker (nm)
30	19.14	3.2
40	25.85	3.8
50	29.7	8.4
60	33.42	13.2
70	37.14	18
80	40.42	23.4

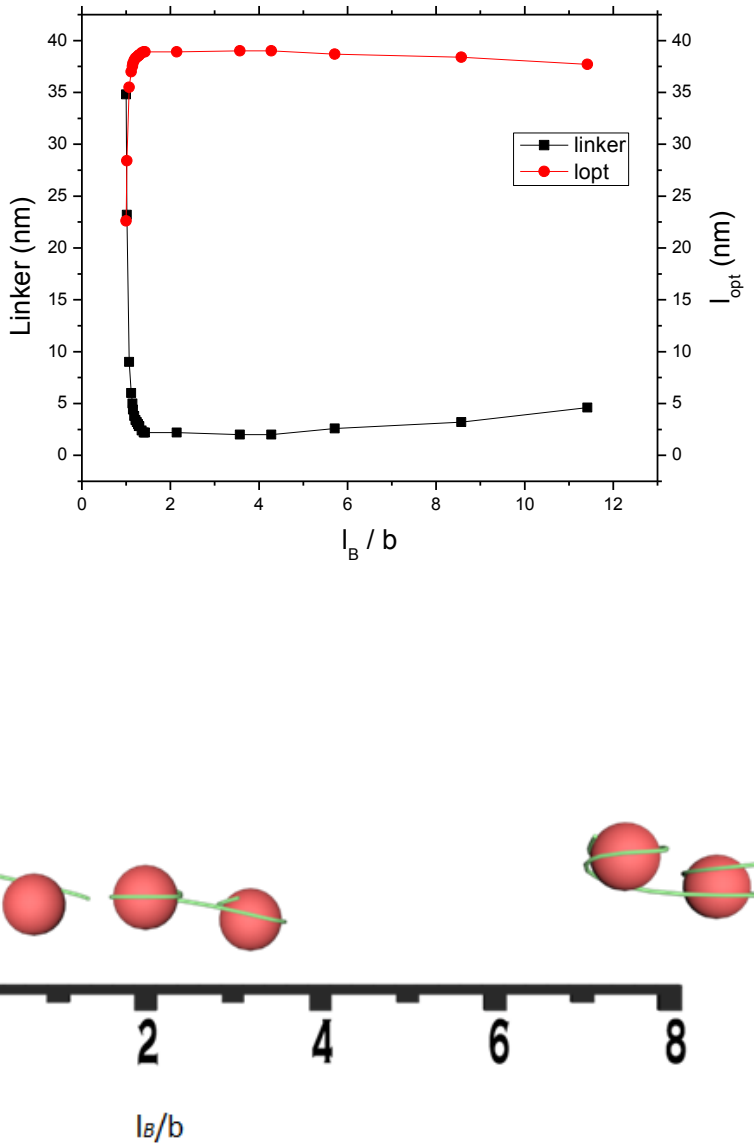
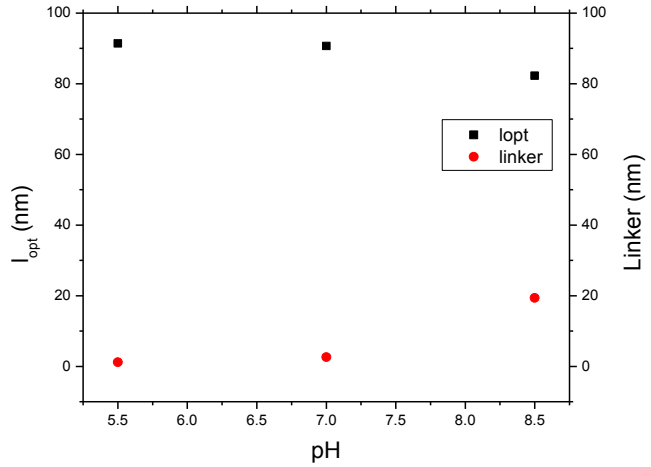


Figure 3.14: Effect of Bjerrum length on linker formed between 2G3 complexes with an flexible LPE chain. The LPE chain has persistence length of 3nm and spacer of 0.7nm, the dendrimers are considered as a penetrable spheres with radius R = 3.2nm.

Table 3.11: Analytical model results from the interaction between 2 G3 dendrimers and LPE chain of length L= 80 nm.

l_B/b (nm)	Linker (nm)	l_{opt} (nm)
1	34.8	22.6
1.014	23.2	28.4
1.071	9	35.5
1.114	6	37
1.142	5	37.5
1.157	4.4	37.8
1.185	3.8	38.1
1.214	3.4	38.3
1.242	3.2	38.4
1.271	3	38.5
1.285	2.8	38.6
1.3	2.8	38.6
1.35	2.4	38.8
1.4	2.2	38.9
1.428	2.2	38.9
2.14	2.2	38.9
3.57	2	39
4.28	2	39
5.71	2.6	38.7
8.57	3.2	38.4
11.42	4.6	37.7



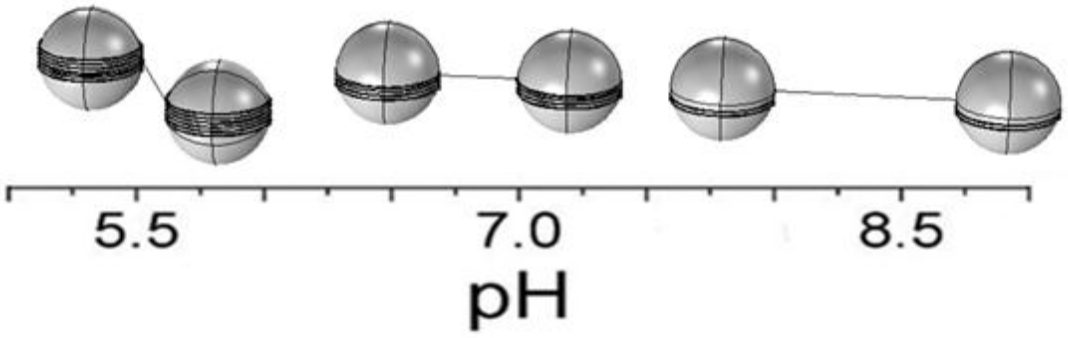


Figure3.15: Linker formed between 2G6 – DNA complexes as a function of pH value. The LPE chain modeled DNA molecules of 184nm, the dendrimers are considered as a penetrable spheres with radius R = 3.5nm.

Table 3.12: Analytical model results from the interaction between 2 G6 ammonia cored dendrimers and LPE chain (541 bps), length L= 184 nm the dendrimers considered as a penetrable sphere with radius R = 3.5nm.

pH value	total charge	l_{opt} (nm)	Linker (nm)
5.5	405	91.4	1.2
7	192	90.7	2.6
8.5	160	82.3	19.4

Chapter Four

Conclusions and Future Work

Chapter Four

Conclusions and Future Work

In this study, we have found that the optimal wrapping length around the dendrimer increased due to the dendrimer generation increase, for both ammonia and ethylenediamine cored. Also as the optimal wrapping length increases, as Bjerrum length increase. Low generations of both cored dendrimers are less affected in the change in Bjerrum length than high generation cored due to the difference in charge and radius value which influence the electrostatic interaction.

We can conclude that when the salt concentration increases at small values of κ, the optimal length increases at higher salt concentration, the optimal length is almost fixed due to a finite length of LPE chain. Whereas for higher generations, there is no significant increase in the optimal wrapping length of LPE chain around dendrimer, indicating that the aggregate formed by the complexation between LPE and dendrimers of higher generations is insensitive to ionic strength. In other words, the aggregate formed from the complexation between LPE chain with dendrimers of higher generation, seems to be more neutralized than those formed of lower generations. This result is in agreement with the experimental study carried by (Carnerup et al., 2011). The effect of salt concentration on the optimal wrapping length depends on the strength of the electrostatic interaction that measured by Bjerrum length l_B.

We can accord the decrease of optimal wrapping length around dendrimer, in all cases as the persistence length of the chain is increased. The effect of chain stiffness is different

from ammonia cored to ethylenediamine cored dendrimers according to the difference in radius, charge value and generations of dendrimer.

The effect between a multiple PAMAM dendrimer- LPE chain complexes, showed that optimal wrapping length increases by decreasing the linker. The total length of LPE chain is divided into two parts, the linker and the optimal wrapping length, so the optimal wrapping length increases with increasing LPE length and linker.

Finally, Dendrimers hold a promising future in various pharmaceutical application and diagnostic field in the coming years, as they posses unique properties, such as higher degree of branching, multivalency, globular architecture and well-defined molecular weight ; thereby offering new scaffold for drug delivery. Also as newer applications of dendrimers will stand out and the future should evidence an increasing number of commercialized dendrimer based for drug delivery systems.

References:

- Ainalem, M. L., Carnerup, A. M., Janiak, J., Alfredsson, V., Nylander, T., (2009), Condensing DNA with poly (amido amine) dendrimers of different generations: means of controlling aggregate morphology, Soft Matter, 5, 2310–2320.
- Ashvini, S., Asheesh, S., Dharmendra, C., Ritu, N., (2012), Dendrimers: A New Class of Nanoscale Polymer for Healthcare System, IJRPS, 2(1), 44-52.
- Boas, U., Christensen, J. B., Heegaared, P. M. H., (2006), Dendrimers in Medicine and Biotechnology: New Molecular Tools, Royal Society of Chemistry, Springer,1-27.
- Buhleier, E., Wehner, W. and Vogtle, F., (1978), Cascade and Nonskid-chain-like Synthesis of Molecular Cavity Topologies, Synthesis, 2,155-158.
- Carnerup, A. M., Ainalem, M. L., Alfredsson, V. and Nylander, T.(2009), Watching DNA condensation induced by poly(amido amine) dendrimers with time-resolved cryo-TEM, Langmuir, 25, 12466–12470.
- Carnerup, A. M., Ainalem, M. L., Viveka, A., Nylender, T., (2011), Condensation of DNA using poly(amido amine) dendrimers: effect of salt concentration on aggregate morphology, Soft Matter, 7, 760–768.
- Cheng, Y., Zhenhua, X., Minglu, M.,Tongwen, X., (2008), Dendrimers as drug carriers: applications in different routes of drug administration, J.Pharm. Sci.,97,123-143.
- Culver, K. (1994). Gene Therapy—A Handbook for Physicians. Mary Ann Liebert, Inc., New York.

- Dootz, R., Cristina T., Pfohl, T., (2011), PAMAM6 dendrimers and DNA: pH dependent “beads-on-a-string” behavior revealed by small angle X-ray scattering.
- Dufe`s, C, F. Uchegbu, I, F., Scha¨tzlein, A, G., (2005), Dendrimers in gene delivery, Advanced Drug Delivery Reviews, 57, 2177– 2202.
- Edelstein, M. L., Abedi, M. R., Wixon, J., and Edelstein, R. M. (2004). Gene therapy clinical trials worldwide 1989-2004—an overview. J Gene Med 6, 597-602.
- Fant, K., Esbjorner, E. K., Lincoln, P., Norden, B., (2008), DNA Condensation by PAMAM Dendrimers: Self-Assembly Characteristics and Effect on Transcription Biochemistry, 47, 1732-1740.
- Franklin, R. E. and Gosling, R. G. (1953). Molecular configuration in sodium thymonucleate. Nature 171:740–741.
- Freeman. W.H., Lodish H, Berk A, Zipursky SL, et al. (2000), Molecular Cell Biology. 4th edition, New York.
- Itaka, K., Kataoka, K., (2009), Recent development of nonviral gene delivery systems with virus-like structures and mechanisms, Eur J. Pharm Biopharm.,71, 475–483.
- Jin, L., Zeng, X., Liu, M., Deng, Y., He, N., (2014), Current Progress in Gene Delivery Technology Based on Chemical Methods and Nano-carriers, 4(3): 240–255.
- Kunze, K. K., Netz, R. R., (2002), Complexes of semiflexible polyelectrolytes and charged spheres as models for salt-modulated nucleosomal structures, physical review E 66, 011918, 1-28.

- Koper, G. J. M., Duijvenbode, R. C. Van, Borkovec. M., (1998), Acid–base properties of poly(propylene imine)dendrimers, Polymer, 39, 2657–2664.
- Lyulin, S. V., Darinskii, A.A., Lyulin, A. V., (2005), Computer Simulation of Complexes of Dendrimers with Linear Polyelectrolytes, Macromolecules, 38, 3990-3998.
- Lee. I, Athey .B. D, Wetzel A, W., Meixner, W., and Baker, J. R., (2002,), Structural Molecular Dynamics Studies on Polyamidoamine Dendrimers for a Therapeutic Application: Effects of pH and Generation, Macromolecules, 35, 4510–4520.
- Manning Q., (1978), Counterion condensation theory, Revs. Biophys. 11, 179-24.
- Maiti, P.K., Bagchi, B., (2006), Structure and Dynamics of DNA-dendrimer complexation: Role of counterions, Water, and Base Pair sequence, Nano Lett., 6, 2478-2485.
- Mishra Ina., (2011), Dendrimer: A nivel drug delivery system,Journal of Drug Delivery & Therapeutics;, 1(2): 70-74.
- Newkome, G. R., Yao, Z. Q., Baker, G. R., Gupta, V. K., (1985), Micelles Part 1. Cascade molecules: a new approach to micelles, J. Org. Chem., 50, 155-158.
- Nguyen, T. T., Shklovskii, B. I., (2001), Overcharging of a macroion by an oppositely charged polyelectrolyte, Physica, A 293, 324–338.
- Örberg, M. L., Schillen, K., Nylander, T., (2007), Dynamic Light Scattering and Fluorescence Study of the Interaction between Double-Stranded DNA and Pol(amido amine) Dendrimers, Biomacromolecules, 8, 1557-1563.

- Park, S. Y, Bruinsma, R. F., Gelbart, W. M., (1999), Spontaneous overcharging of macro-ion complexes, Europhys. Lett., 46,454.
- Paulo,P. M. R, Lopes, J. N.C. and Costa, J. S. M.B. (2007), Molecular Dynamics Simulations of Charged Dendrimers: Low-to-Intermediate Half-Generation PAMAMs, Phys. Chem. B, 111, 10651–10664.
- Pushkar S, Philip A, Pathak K and Pathak D, (2006), "Dendrimers: Nanotechnology Derived Novel Polymers in Drug Delivery", Indian J. Pharm. Educ. Res., 40 (3),153-158.
- Qamhieh, K., Nylander, T., Ainalem, M. L., (2009), Analytical Model Study of Dendrimer/DNA Complexes, Biomacromolecules, 10, 1720 -1726
- Qamhieh, K., Abu Khaleel, A., (2011), Analytical model study of complexation of Dendrimer as an ion penetrable sphere with DNA, J. of colloids and surfaces, 442, 191– 198.
- Qamhieh, K., Nylander, T., Black, C, F., Attard, G, S., Dias, R, S., Ainalem, M .,L, (2014), Complexes formed between DNA and poly(amido amine) dendrimers of different generations modelling DNA wrapping and penetration, the Owner Societies, 16, 13112—13122.
- Rachael R., (2013), DNA: Definition, Structure & Discovery.
- Rosenberg, S. A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R. A., Moen R., Karson, E. M., Lotze, M. T., Yang, J. C., and Topalian, S. L. (1990). Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 323, 570-78.

- Schiessel, H., Bruinsma, R. F., Gelbart, W. M., (2001), Electrostatic complexation of spheres and chains under elastic stress, J. of chemical physics, 115, 7245-7252.
- Tambe, V,R., Pakhare, S, S., Jadhav, M, G., Tiwari, S, S., Rai, C,R., (2012), Dendrimer: A Smart Polymer, IJRRPAS, 2(3).513-528.
- Tomalia D.A, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J and Smith P, (1985), A New Class of Polymers: Starburst-Dendritic Macromolecules, Polym. J., 17(1) , 117-132.
- Tomalia, D. A. and J.M.J., Fréchet,J. M. J. and Wiley & Sons, J. Ltd., Chichester (2001) "Dendrimers and Other Dendritic Polymers .
- Tomalia, D. A., (2010), Dendrons / dendrimers: quantized, nano-element like building blocks for soft-soft and soft-hard nano-compound synthesis, soft matter, 6, 456-474.
- Vedha Hari,B. N., Kalaimagal, K., Porkodi, R., Gajula, P, K., Ajay, J, Y., (2012), Dendrimer: Globular Nanostructured Materials for Drug Delivery, 4, 1, pp 432-451.
- Watson, J., Crick, F., (1953), Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid, Nature, 171(4356), 737-738.
- Welch,p., M. Muthukumar, (2000), Dendrimer-Polyelectrolyte Complexation: A model guest-host system, Macromolecules, 33 (16), 6159-6167.
- Zinchenko, A. A., Chen, N., (2006), Compaction of DNA on nanoscale three-dimensional templates, J. Phys.: Condens. Matter,18, R453.

الملخص

بينت هذه الدراسة كيفية حدوث عمليه التجمع بين مركب الدندريمير والحمض النووي باستخدام كرة قابلة للاختراق من قبل الايونات المحيطة , (Ion penetrable sphere) وتمثلت هذه الدراسة باستخدام نظامين مختلفين من الدندريمير باختلاف نوع النواة والاجيال منها الامونيا بأجيال (G1) و (G2)و (G3) و(G4)و(G6) والايثيلين بأجيال مختلفة (G1) و (G2) و (G3) و (G4)و (G5) و (G6) و (G7)و (G8) و (G9) و (G10) باستخدام نموذج نظري تم تصميمه من قبل (Qamhieh,et al,2009)يصف التفاعل بين ال (Dendrimer) و(LPE chain).

ولقد وجدت خلال هذه الدراسة ان درجة الالتفاف (l_{opt}) ال (LPE chain)حول الدندريمير باختلاف كلا من النظامين و اجيالهم تتأثر بالزيادة من خلال زيادة كل من الاجيال,

وطول (LPE chain), وتركيز الاملاح في المحلول, وتقل هذه الدرجة بزيادة صلابةال (LPEchain).

وقد تم دراسة تأثير تغير طول ال (LPE chain) مع اثنين من الدندريميرعلى درجة الالتفاف (l_{opt}) بترابط يطلق عليه اسم (Aggregate) وعليه يتكون مايعرف بال (Linker) وقد تبين من خلال هذه الدراسة ان زيادة طول ال (LPE chain) تزيد من درجة الالتفاف (l_{opt}) ويقل تكون ال (Linker).