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Analytical Study of Dendrimer / DNA Complexes

Amani Basem Mahmoud Sabbah

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Prepared by:

Amani Basem Mahmoud Sabbah

B.A. Life Science, 2007, Al- Quds University

Supervisor: Dr. Khawla Qamhieh

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Prepared by: Amani Basem Mahmoud Sabbah

Registration No: 20812539

Supervisor: Dr. Khawla Qamhieh

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The name and signature of examining committee member are as follows:

1- Head of the committee: Dr. Khawla Qamhieh Signature: .....

2. Internal Examiner: Prof. Dr. Ibrahim Kayali Signature: .....

3. External Examiner: Dr. Jamal Ghabboun Signature: .....

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Dedication

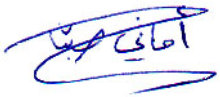
I dedicate this thesis to the sun of life my family, especially, to my mother and father for their patience and continuous support ---- to my wonderful husband and brothers for their help and understanding.

Declaration

I hereby declare that this thesis is based on the results found by myself. Materials of works found by other researchers are mentioned by references. This thesis, neither in whole or in part, has been previously submitted for any degree.

The work was done under the supervision of Dr. Khawla Qamhieh, at Al – Quds University, Palestine.

Name: Amany Basem Mahmoud Sabbah

Sign: 

Date: 30/12/2013

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My thanks extend to my family, my husband, friends, who stood beside me and encouraged me constantly.

Finally, I wish to express my gratitude to the Almighty Allah for providing the grant to make this thesis possible.

Amani Basem Sabbah

Abstract

The complexation of negative charge linear polyelectrolyte chain (LPE) and positive charge dendrimers of ammonia cored of generations G2,G3,G4 and G6 and ethylenediamine cored of generations G2,G3,G4,G5 and G6 has been studied by applying a new penetrable sphere model developed by Qamhieh and coworker for complexation of ion-penetrable sphere with (LPE) chain. Different factors such as the strength of electrostatic interaction represented by Bjerrum length, salt concentration, persistence length, chain length and charge of dendrimer that control the formation and behavior of LPE-dendrimer complex have been investigated.

For the complexation of one dendrimer with one LPE chain, it is found that the wrapping degree of the chain around the dendrimer increases by increasing Bjerrum length, salt concentration, chain length, dendrimers charge, and decreases by increasing the rigidity of the chain (persistence length).

The degree of these effects on complex formation depends on the generation and on the dendrimer cored type (ammonia or ethylenediamine cored dendrimers. For the complexation of two dendrimers with one LPE chain it is found that the contraction of dendrimer during interaction between dendrimers and LPE chain in the complex increases the optimal wrapping length and decreases the linker length. This result is in agreement with the previous result of Qamhieh and co-workers studies for other generations of dendrimers.

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List of Abbreviations

DNA	Deoxyribonucleic acid
LPE	Linear Polyelectrolyte
PAMAM	Polyamidoamine
N_{ch}	Number of monomers on the chain
Z_{dend}	Number of functional groups of dendrimer
EDA	Ethylenediamine
Bp	Base-pair
l_{opt}	The optimal wrapping length of chain around dendrimer
l_{iso}	The length of the chain needed to neutralized the charge of dendrimer
L_B	Bjerrum length
l_p	The persistence length of LPE chain
DLS	Dynamic light scattering
Cs	Salt concentration
dsDNA	Double strand DNA
ssDNA	Single strand DNA
G	Generation of dendrimer
UV-VIS	Ultra-violet-visible spectroscopy
LLS	Laser light scattering
CG	Coarse-grained
MD	Molecular dynamics simulation
G	Guanine
A	Adenine
C	Cytosine
T	Thymine

Chapter One

Introduction

Chapter One

1.1 Introduction

In 1952 Hershey and Chase made the groundbreaking discovery that DNA was the genetic material. Within the next decade, the structure of the deoxyribonucleic acid (DNA) was discerned, followed by many experimental and analytical studies that focus on understanding and studying the structure, function and characteristics of DNA. This lead to the birth of a new field of molecular biology that focuses, on studying everything related to the genetic material. Deoxyribonucleic acid (DNA) is the nucleic acid that carries the genetic material which controls all function inside living cells. This component works as code for inherited information. All this discoveries and studies about DNA have opened the door for the idea of gene therapy.

Gene therapy can be described as the intracellular delivery of genetic material to generate a therapeutic effect by correcting an existing abnormality or providing cells with a new function. Wide range of chronic diseases, especially those caused by inherited single gene mutation are candidates for gene therapy including diseases such as cancer, vascular disease, neurodegenerative disorders and other acquired diseases that considered as targets for gene therapy. The basic principle of gene therapy in simple words is to deliver the target gene into the human cell to get the required result. The process mainly relies on the transport mechanism of the target gene inside the cell. There are many possible ways to deliver the gene. The traditional mechanism of gene delivery was the viral system, there are certain types of virus used as carrier for genetic material such as Retrovirus and Adenovirus. Despite the ability of the viral system to carry the gene into a specific target cell, it usually possesses very high transfection efficiency, leading to high gene expression rates. It also has many defects such as the high immune response, its toxicity for human body and it also doesn't protect the gene from DNase enzyme which means that the gene stays for a very short period inside the cell. Because of these side effects the need for alternative to the viral system was growing. The alternative to the viral strategies has been the application of chemically synthesized vehicles such as liposome or the use of naked plasmid DNA, or the use of certain types of natural and synthetic polymers such as (Dendrimers).

Although experiments have provided vital information on the large scale interactions between dendrimers and other molecules, many atomic-level questions that cannot be answered by experiments remain to be solved. Therefore, theoretical and computational modeling methods have been applied to investigate the atomic-scale insights into the interactions of dendrimers with other molecules. Initially, these consisted of Monte Carlo and Brownian dynamics simulations, but recent advances in computer speed and simulation methods have made it possible to study dendrimers and their interactions with explicit solvents and other molecules by atomistic and
coarse-grained (CG) molecular dynamics (MD) simulations.

There are several factors that control the DNA / dendrimer complex relation such as the charge of the sphere $eZ(l)$, the linear charge density of the polymer $-e/b$, the ionic strength, the sphere radius R, the flexibility of the polymer, the number of dendrimer in the complex, type and generation of the dendrimer used, and the pH number. There are several theoretical studies and computer simulations that have been done on this field to increase the understanding of the relation between DNA and dendrimer, and also to clarify the mechanism of releasing DNA inside the cell:

Qamhieh, Nylander, and Ainmalen(2009) studied the interaction between positively charged poly(amido amine) (PAMAM) dendrimers of generation(G 4) and DNA in two different lengths using a theoretical model designed by Schiessel for a semiflexible polyelectrolyte and hard spheres. The model was modified to take into account that the dendrimers be regarded as soft spheres, which means that the radius is not constant when the DNA interacts with the dendrimer. By this model they determine the charge of the complex as well as how much of the DNA chain is wrapped around the dendrimers. They provided further understanding of the formation and the structure of dendrimer/DNA complexes by using a theoretical model of the complex formation.

Örberg et al (2007), conducted a series of experiments by different mechanism, and as a result of these experiments they proposed a binding model for discrete aggregates formed between DNA and PAMAM dendrimers of generation (G 4). The complex formation process was found to be cooperative,(see Figure1.1) showing the coexistence of both condensed DNA and free DNA in its native form.

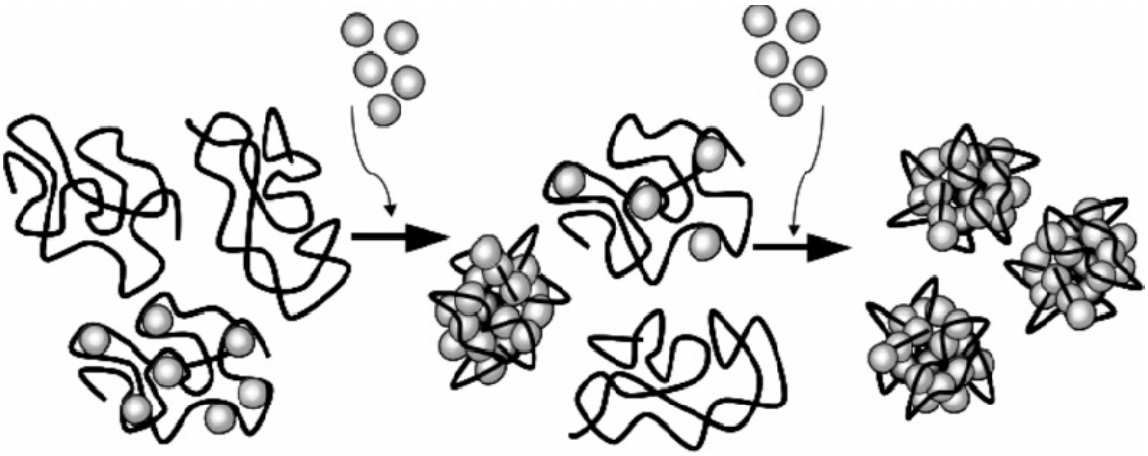

Figure 1.1: The proposed binding model for discrete aggregates formed between DNA and PAMAM dendrimers of generation 4 (Örberg et al ., 2007).

Ainalem et al (2009), studied the morphology of dendrimer/DNA aggregates as a function of the dendrimer generation, i.e. the size, total charge and charge density, to provide further information about the condensation process. By using a monodisperse DNA sample of 4331 base pairs (bp), dendrimers of generation 1, 2, 4, 6 and 8 (G1, G2, G4, G6 and G8) and three non-invasive experimental techniques(Cryogenic transmission electron microscopy, Dynamic light scattering, and steady state fluorescence spectroscopy). The study showed that the morphology of the aggregates transition from rods and toroids to globular aggregates with increasing dendrimer generation, i.e. surface charge density.

Debye-Hückel, performed Monte Carlo and Brownian dynamics simulations to study the electrostatic interaction potential (electrostatically screened Coulomb potential), where the effects of counterions and solvents were treated implicitly.

Welch and Muthukumar performed the first Monte Carlo simulations of the interactions between a cationic dendrimer (G4-G6) and an anionic polyelectrolyte. They showed that a dendrimer can encapsulate or interpenetrate a polyelectrolyte chain, or display a unique "chain-walking" phenomenon. They also predicted that the degree of polyelectrolyte encapsulation significantly depends on the salt concentration, size, and charge density of the dendrimer and polyelectrolyte.

Lyulin et ,al. used Brownian dynamics simulations to study the complex formed by a cationic dendrimer (G1-G4) with a sufficiently longer anionic polyelectrolyte chain (consisting of 24 to 90 negatively charged monomers). He noted that when the linear chain and the dendrimer had the same number of charges, the chain wrapped around the surface of the dendrimer. But by

longer chain that had more charges, more of the chain was adsorbed into the dendrimer than was necessary for dendrimer neutralization (overcharging).

Maiti and Bagchi performed 20 ns-long atomistic MD simulations of G2-G4 dendrimer-ssDNA complexes in explicit water. Their work showed that under some circumstances the degree of over-compensation is very limited. Also during his work in dendrimer of G4 that has enough positive charge (64) to neutralize the 37 charges on the ssDNA they noted that DNA first wrapped around the dendrimer then after a period of time it penetrated inside the dendrimer which made dendrimer swollen (see figure 1.2).

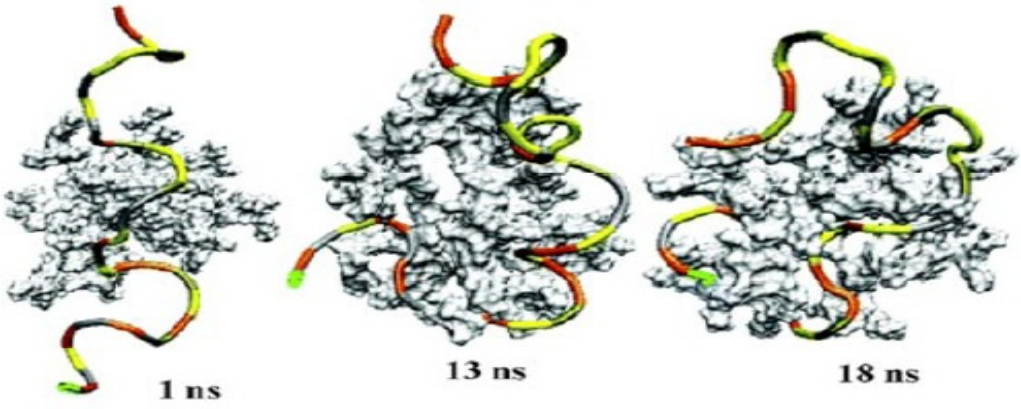

Figure 1.2: Snapshots every few ns of formation of DNA-dendrimer complex (Maiti and Bagchi, 2006).

Wallin and Linse performed a series of Monte Carlo simulations including determination of the free energy of the formation of complexes between dendrimer and DNA.

H. Scheissel, Marky, and Manning have studied complexes between semiflexible polyelectrolyte and a rigid sphere using an extremely simplified model for the electrostatic interaction. They showed that there could exist a wrapping transition between a slightly bent conformation of the polyelectrolyte close to the sphere and a conformation where the polyelectrolyte wraps around the sphere.

1.2 General characteristics of DNA

DNA exists in many possible conformations that include A-DNA, B-DNA, and Z-DNA forms, although only B-DNA and Z-DNA have been directly observed in functional organisms (see Table1.1), and the most abundant form found in nature is B-form (see Figure1.3).

Table 1.1: A comparison of the structural properties of A, B and Z DNAs as derived from single-crystal X-ray analysis (Dickerson et al, 1982).

	Helix Type		
Properties	**A**	**B**	**Z**
Overall proportions	Short and broad	Longer and thinner	Elongated and slim
Rise per base pair	2.3A°	3.32A°	3.8°
Helix-packing diameter	25.5A°	23.7A°	18.4 **A°**
Helix rotation sense	Right-handed	Right-handed	Left-handed
Base pairs per helix repeat	1	1	2
Base pairs per turn of helix	≈11	≈10	12
Rotation per base pair	33.6°	35.9 °	- 60°per 2 bp
Pitch per turn of helix	24.6A°	33.2A°	45.6A°
Tilt of base normals to helix	+ 19°	- 1.2°	- 9°
Base-pair mean propeller twist	+ 18°	+ 16°	≈ 0°
Helix axis location	Major groove	Through base pairs	Minor groove
Major-groove proportions	Extremely narrow but very deep	Wide and of intermediate depth	Flattened out on helix surface
Minor-groove proportions	Very broad but shallow	Narrow and of intermediate depth	Extremely narrow but very deep
Glycosyl-bond conformation	Anti	Anti	anti at C, syn at G

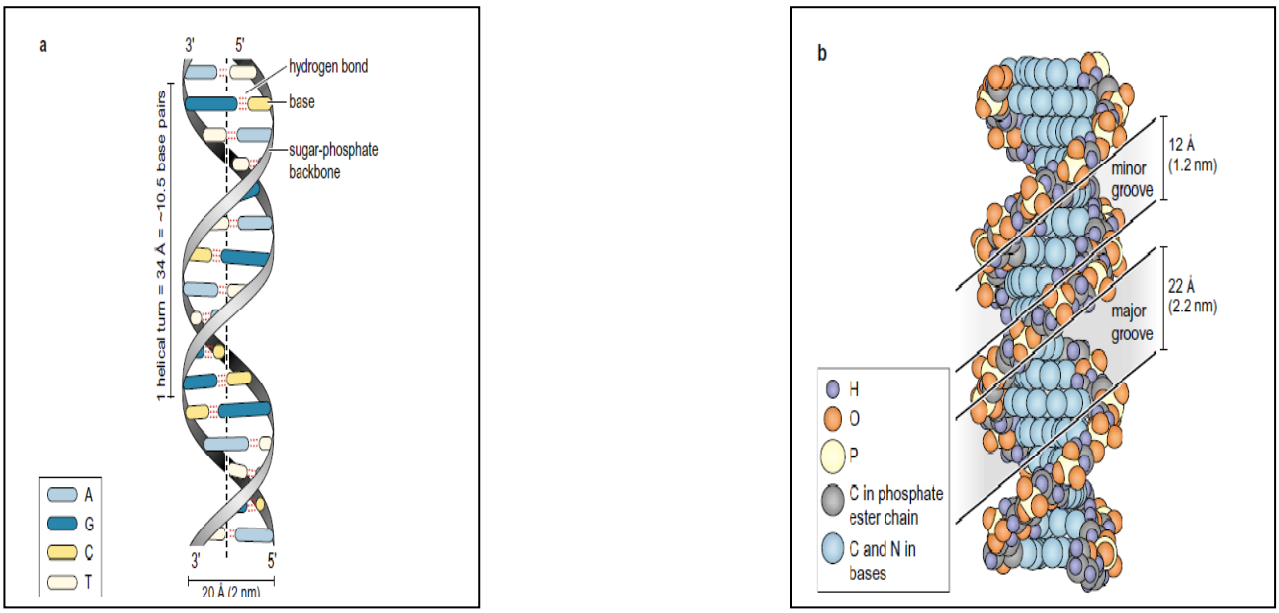

Figure 1.3: The Helical structure of B-form DNA, (a) Schematic model of the double helix, one turn of the helix (34 A□ or 3.4nm), 10.5 base pairs, (b) Space-filling model of the double helix (Dickerson et al, 1982).

The structure of all DNA forms is composed of two helical chains each coiled around the same axis. The double helix comprises of two tightly associated polymer chains. These chains are anti-parallel, running in opposite direction. Each chain consists of a building unit known as nucleotides(see Figure 1.4.a). A nucleotide is composed of a five-carbon sugar called 2-deoxyribose that bond with phosphate group that carry a negative charge

making DNA strand a polyelectrolyte, and bonds with a nitrogen base called nucleobase. The nitrogen base are classified into two types (a) Purines which are heterocyclic aromatic organic compound that has two carbon-nitrogen rings, includes adenine(A) and guanine(G), (b) Pyrimidines are the same as purines but they only have one carbon-nitrogen ring, includes cytosine (C) and thymine(T), this A.T and G.C pairing is known as Watson and Crick base pairing(see Figure1.4.b).

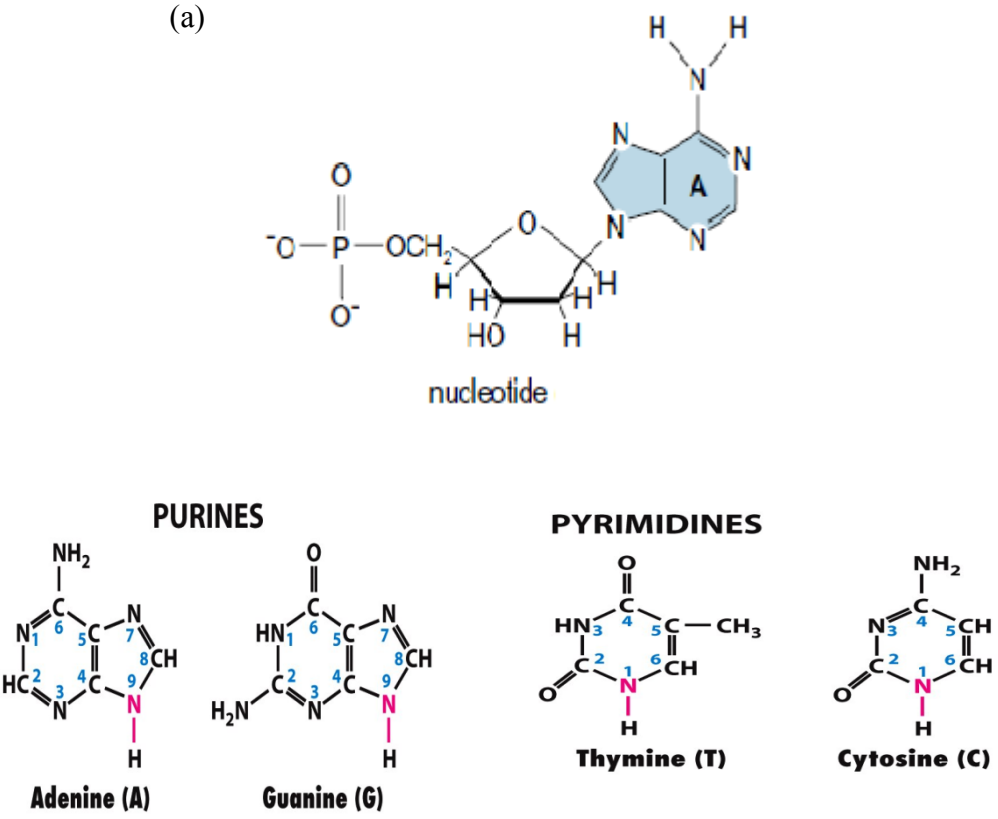

Figure 1.4: (a) The structure of a nucleotide, (b) nitrogen base purines and pyrimidines that forms the nucleic acids.

The major bond that hold the double helix together is the hydrogen bond between the complementary nitrogen base on opposite strands that forms the base pair, adenine base pair with thymine by two hydrogen bonds, cytosine base pair with guanine by three hydrogen bonds, which lie horizontally between the two spiraling strands. The length of each nucleotide is approximately 0.34nm. It seems that each individual repeating building unit is very small, but if we look at the all DNA polymer it will be very large composed of millions of nucleotides. For example chromosome number 1 in humans consists of approximately 220 million base pairs and is 85 mm long, this leads to the question how DNA with its large size is found inside the cell??

In eukaryote cells, DNA is complexed with proteins called histones, enabling large genomes to fit inside the cell nucleus. Electrostatic attraction that forms between the positively charge amino acids in histones and negative charge of phosphodiester backbone of DNA, allows DNA to wrap around the histone. This attraction lead to form a structure called chromatin which makes liner DNA compacted inside the cell (see Figure1.5).The relation between the DNA and histone helps in the born the idea of DNA chemical vector such as dendrimers that have many applications in gene therapy.

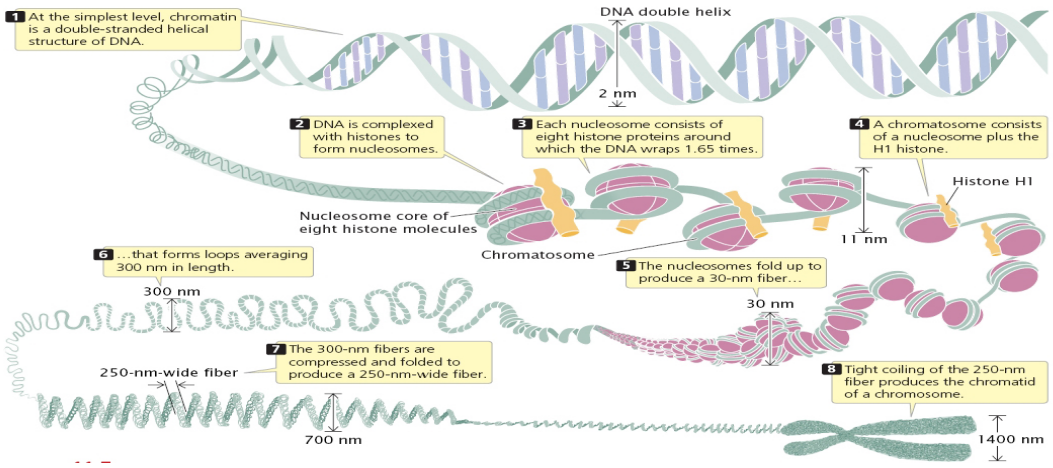

Figure1.5: DNA condensation by histone inside the cell.

1.3 Dendrimers

Dendrimers are a new class of polymeric materials, they are macromolecules with a regular, highly branched, three-dimensional, multivalency, globular, perfect monodisperse architecture and well-defined molecular weight, that resemble biomolecules (i.e., proteins). Dendrimers are considered as a sub-group of hyper-branched polymers, and they have often been referred to as the “Polymers of the 21st century”.

In 1978 the first successful attempt to create and design dentritic structure by organic synthesis was carried out by V□gtle and co-workers and the structure was named " cascade molecules ". And in 1980 another group lead by Tomalia relying on V□gtle cascade molecules developed a new class of cascade polymers called " dendrimers " built up from two Greek words " dendros " meaning " tree " or " branch " and " meros " meaning " part ". At the same time, Newkome's group independently reported a synthesis of similar macromolecules, called " arborols " from Latin word " arbor " also meaning a tree. In 1988 another dendritic structure was developed by J.P.Tam called " Multiple Antigen Peptide".

Because of their special and unique structure, properties and wide range of applications, and after synthesizing them, focusing on and studying of dendrimers exponentially increase, and it has become a special chemistry research field.

1.3.1 Dendrimers Structure:

Dendrimer are complex molecules with very well defined chemical structure. They consist of three major architectural components core, branches, and end groups (see figure1.6):
The core which consists of a molecule containing two or more functional groups such as a nitrogen atom that acts as starting unit, from which branches emanate producing a radial structure. Each branch is a repeating unit composed of atoms such as carbon and other, that are added to the core by a repeating series of chemical reactions, each branch forms a layer around the core called " generation (G) ".The end groups are the exterior layers that carry the functional group.

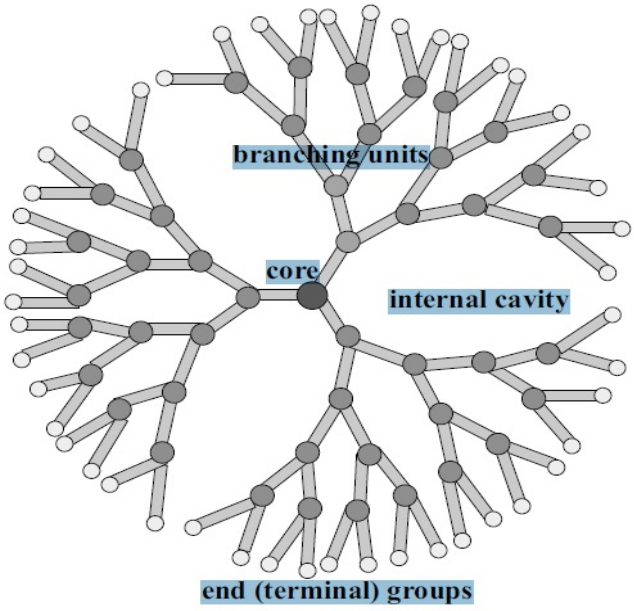

Figure 1.6: Spherical macromolecular structure of the fourth generation dendrimer (G4) (Klajnert et al., 2001).

The growth of dendrimers is exponential, the increase in both molar mass and number of terminal groups can be predicted mathematically (Tomalia et al.1990).
The molar mass can be calculated by :

$$M = M_c + n_c .[M_m.(n_m G-1/ n_m-1) + M_t . n_m G],$$

Where: M_c- is the molar mass of the core, M_m- the molar mass of the branched monomer, M_t- the molar mass of the terminal groups, n_c- the core multiplicity, n_m- the branch-

juncture multiplicity, G- the generation number. And the increase of the number of dendrimer terminal groups (Z) is consistent with the geometric progression:

$Z = nc \cdot n_m G$

Table 1.2 Theoretical properties of ($G_0 – G_{10}$) for PAMAM dendrimer.

Generation (G)	Ammonia cored dendrimers		Ethylenediamine cored dendrimers	
	Molecular mass	Number of terminal groups	Molecular mass	Number of terminal groups
0	359	3	516	4
1	1043	6	1428	8
2	2411	12	3252	16
3	5147	24	6900	32
4	10619	48	14196	64
5	21563	96	28788	128
6	43451	192	57972	256
7	8722	384	116340	512
8	17479	68	233075	1024
9	349883	1536	466548	2048
10	700091	3072	933492	4096

1.3.2 Dendrimer synthesis:

The synthesis used for dendrimer preparation permit almost entire control over the critical molecular design parameters such as size, shape, surface/interior chemistry, flexibility, and topology. Many dendrimer synthesis rely upon traditional reactions, such as the Michael reaction or the Williamson ether synthesis whilst others involve the use of modern techniques and chemistry, such as solid-phase synthesis, organo-transition-metal chemistry, organosilicon chemistry, organo-phosphorus chemistry, or other contemporary organic methodologies. The choice of the growth reaction dictates the way in which the branching should be introduced into the dendrimer.

Dendrimers are synthesized using divergent or convergent methods of growth. In divergent growth, the construction of dendrimer takes place in a stepwise manner starting from the core that reacts with a monomer containing one reactive functional group and two inactive groups, leading to the first generation dendrimers. As this step is repeated, a new layers or generations are building up, (see figure1.7). The first dendrimers made by divergent synthesis approaches by V□gtle in 1978. There are two major defects in using divergent growth especially in higher dendrimer generation, firstly, the purification process of the desired product from the reactants is very hard because of the similarity between them and the by-products. Secondly, the number of reaction points increases rapidly throughout the

synthesis of dendrimers, which can cause trailing generation (loss of dendrimer branches and wedges) especially with higher generations.

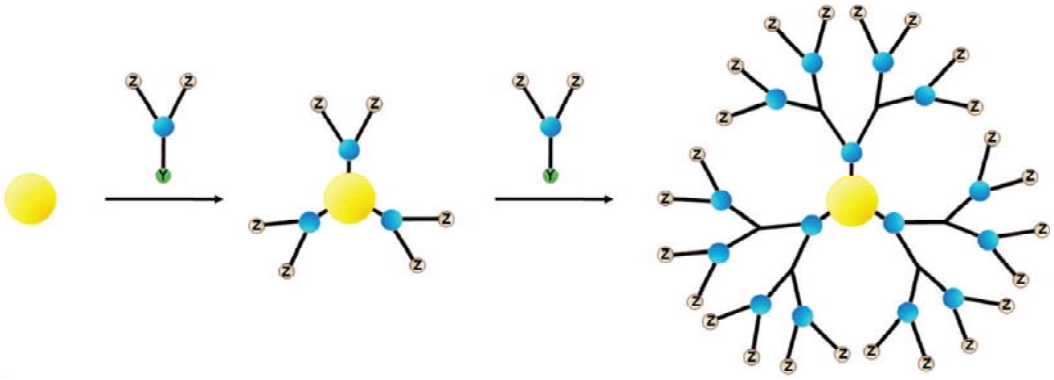

Figure 1.7: Schematic drawing showing the divergent method for synthesis of dendrimers. It starts with a multifunctional initiator core (yellow) that reacts with the chemically activated focal point (Y) of a branched monomer (blue) to produce the first-generation dendrimer. Higher generations are synthesized by the iterative addition of the branched monomers, producing a complete dendrimer terminated with functional chemical groups (Z) (Medina et al., 2009).

In 1990 a convergent synthetic approach was introduced by Jean Fréchet. In convergent growth construction of dendrimers take place from the surface and inwards towards the core, by mostly "one to one" coupling of monomers thereby creating dendritic segment, dendrons, of increasing size as the synthesis progresses (see figure 1.8). Convergent growth method has several advantages. It is relatively easy to purify the desired product, and the final resulting dendrimer is more monodisperse than by the divergent growth.

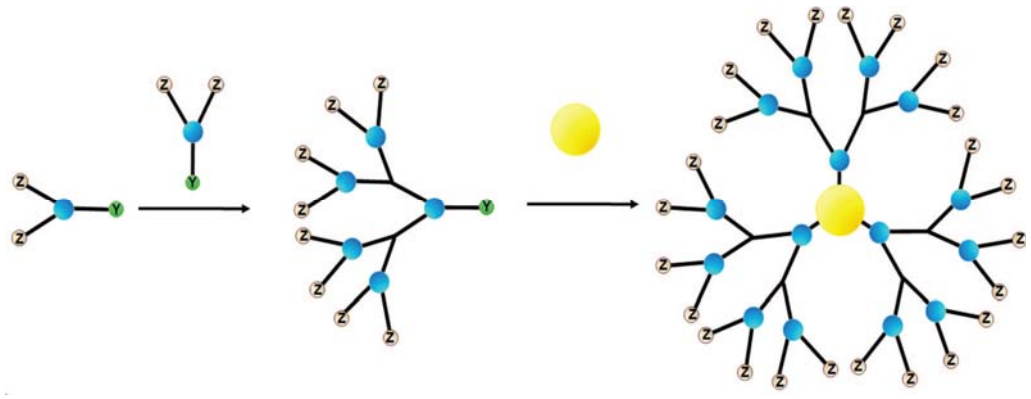

Figure 1.8: Schematic drawing showing the convergent method for synthesis of dendrimers. The dendrimer surface is formed by reaction of the chemically active focal point (Y) of the branched monomer to the functional groups (Z) of another monomer. Dendrons grow by iterative coupling of monomer units to the parent dendron until the desirable dendron size is reached followed by coupling the dendron's focal point to a multifunctional initiator core (yellow) to produce the complete dendrimer (Medina et al., 2009).

There are many types of dendrimers such as "Multiple Antigen Peptide dendrimers" MAP-dendrimers, but the most popular types of dendrimers are polyamidoamine (PAMAM) and polypropyleneimine (PPI). Despite difficulties in synthesis and the high cost of dendrimers, these two types of dendrimers are manufactured on a rather large scale, polyamidoamine (PAMAM) are commercially available up to generation 10(G10) by the Aldrich Chemical Company and Dendritech and polypropyleneimine (PPI)are commercially available up to generation 5(G5) by Aldrich and DSM Fine Chemicals**.**

1.3.3 Properties of dendrimers:

Dendrimers are macromolecules with nanoscale sizes range from 1
- 100 nm, that has similar dimensions to important bio-building blocks, e.g., proteins, DNA. The diameter of the dendrimer increases exponentially as the generation of dendrimer increases, e.g., ammonia cored dendrimer of generation G0 has a diameter of 1nm and G6 has a diameter of 6.7nm. Also the shape of dendrimer changes as the generation changes, i.e. lower generation adopts a more open planar-elliptical shape with transition to a more compact spherical shape for higher generation, see figure 1.9.

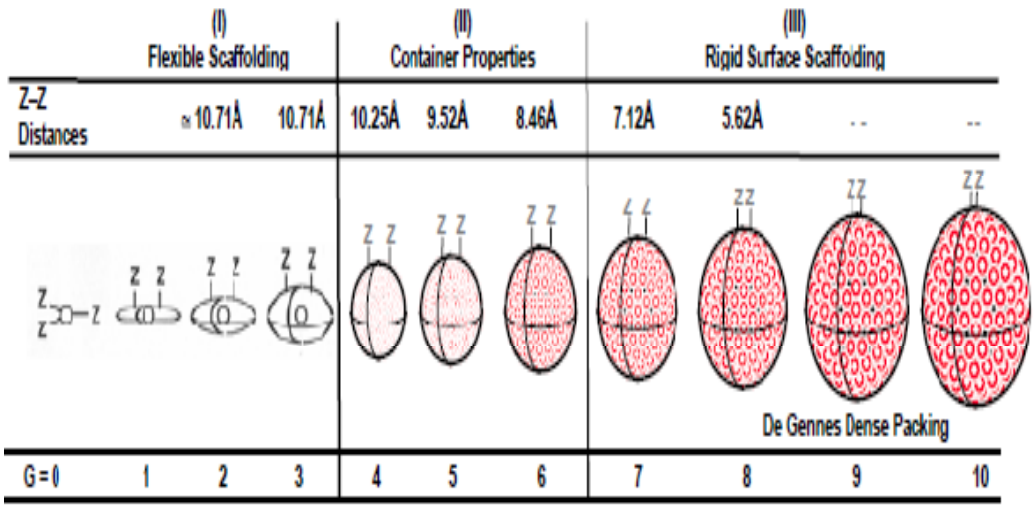

Figure1.9: Shape of dendrimers at different generations (Tomalia, 2010).

Dendrimers have significant structure, the presence of many terminal group is responsible for high solubility and miscibility and high reactivity in comparison with liner polymers, i.e., being polyvalence. The solubility of a dendrimer depends on the nature of the surface group found in the outer layer, that can be hydrophilic or hydrophobic end group. And the

presence of internal cavities in their structure makes them good candidates for using as vectors for drug or genetic material.

Dendrimers are monodisperse macromolecules, the size and molecular mass of dendrimers are well-defined and can be specifically controlled during the synthesis process. Many techniques are used to study the chemical and physical properties of dendrimers such as, ultra-violet-visible spectroscopy (UV-VIS) that is used to monitor the synthesis process of dendrimers, and electrochemistry gives information about the possibility of interaction of electro active end groups. Laser light scattering (LLS) determines the hydrodynamic radius of dendrimers, and many others experimental methods are used to investigate the dendrimers characteristics.

There are several factors that control the structure, behavior and reactivity of dendrimers such as pH value, type of solvent and salt concentration depending on the type of terminal group. For example, PAMAM dendrimer with ammonia cored become more condense and has spherical structure as the value of pH increase. All these factors affect the application of dendrimers and help control their size and shape.

1.3.4 Application of dendrimer

As a result of their unique structure and behavior, dendrimers are suitable to be used as nanocarriers for a wide range of biomedical and industrial applications such as drug delivery, gene delivery, tumor therapy, diagnostics, etc.

Dendrimers are considered to be ideal functional building blocks for the creation of nanostructure due to their self-assembly properties (Fréchet 1994, Zimmerman et al.1996). Some researchers (Newkome et.al.1991; de Brabander-van den Berg, et.al.1995; Stevenlmans, et al.1996) introduced the idea of a dendrimer box, due to their ability to encapsulate certain molecules, so they could be used as vehicles for delivering genetic materials or drugs into cells.

The ability to control the dendrimer size and shape makes it a good strategy to be used as a carrier of DNA to the target cell in gene therapy. There are several experiments that prove the validity of using dendrimer as carrier of DNA and study the relation between the dendrimer and DNA in the complex form. In 2007 Örberg, et al, used different instruments and techniques such as dynamic light scattering, fluorescence spectroscopy and gel

electrophoresis. They examined a sample of dendrimer PAMAM(G4) with salamon sperm DNA diluted in solution of NaBr. They examined this stock solution in different concentrations and proved the ability of using dendrimer as carrier for DNA.

1.3.5 PAMAM dendrimer

PAMAM dendrimers refers to polyamidoamine dendrimers which are also known as starburst dendrimer. They are synthesized by divergent growth starting from ammonia(NH_3) or ethylenediamine(EDA) initiator core reagent(see figure1.10), the first synthesized was reported by Tomalia and co-workers in the mid-1980s. Because of their well-defined size and molecular mass, high positive charge, high water solubility and low toxicity it became the most widely studied type of dendrimers and have been commercially supplied by Dendritech, Inc. since 1994 into both industrial and biomedical applications, and they are available up to generation 10, usually as methanol solution. Due to their unique properties, the PAMAM dendrimers have the longest term of successful commercial applications, and are used in many fields such as in vitro diagnostic, drug delivery and additive in ink formulation.

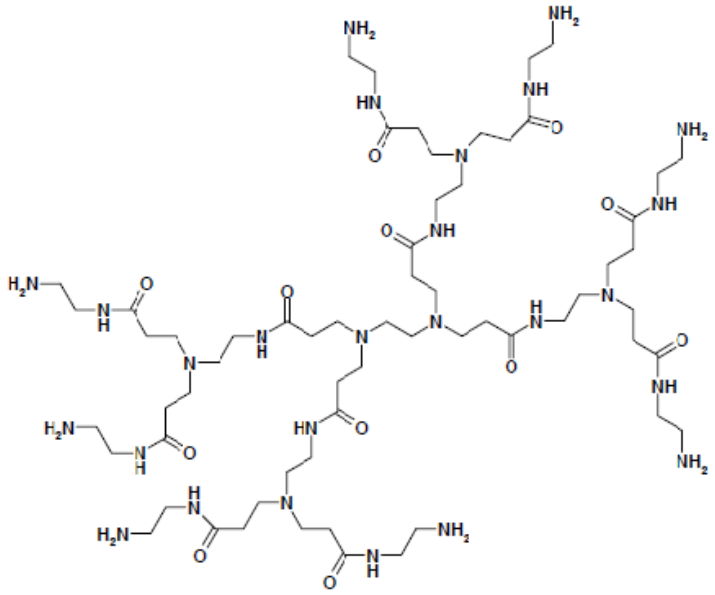

Figure1.10: A G2 PAMAM dendrimer with an ethylenediamine core and 16 functional primary amine groups (Ainalem et al., 2011).

Due to the positive charge they carry, the PAMAM dendrimers is an excellent candidate to be used as vector in gene therapy, and its ability to bind with DNA and facilitating its entry inside the cell through the negative cell membrane, and it forms a complex with DNA which protects DNA from the cell enzymes. Also it has low toxicity which means

less immune reposed, and prolonged period of translation without being degreed. Seib's studies (2003) using laser confocal microscopy, and high-resolution transmission electron microscopy proved that DNA and dendrimer form a complex support the entrance of DNA to the cell (see figure1.11).

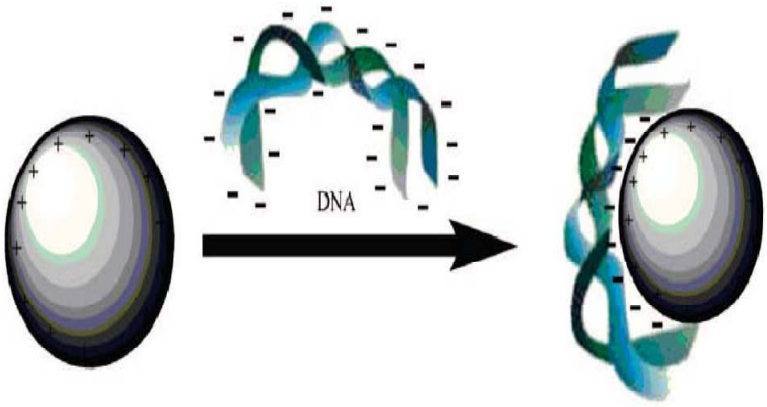

Figure 1.11: Schematic diagram showing formation of dendrimer-DNA complex mediating charge interaction between positively charged dendrimer and negatively charged DNA (Doshi, 2011).

1.4 Complex formation between DNA and PAMAM dendrimers

In order to deliver the effective-DNA into the cell it must be protected from cell enzyme such as DNase that digest DNA, so DNA can't be delivered as free-necked DNA, the formation of DNA- dendrimer complex provides the protection of DNA from degradation. The control role in the formation of this complex is the electrostatic interaction between the positive charge dendrimer and negative charge DNA, but in order to be an efficient way to deliver the DNA inside the cell there are many factors that control this process such as the charge of dendrimers, pH value etc,(see figure1.12).

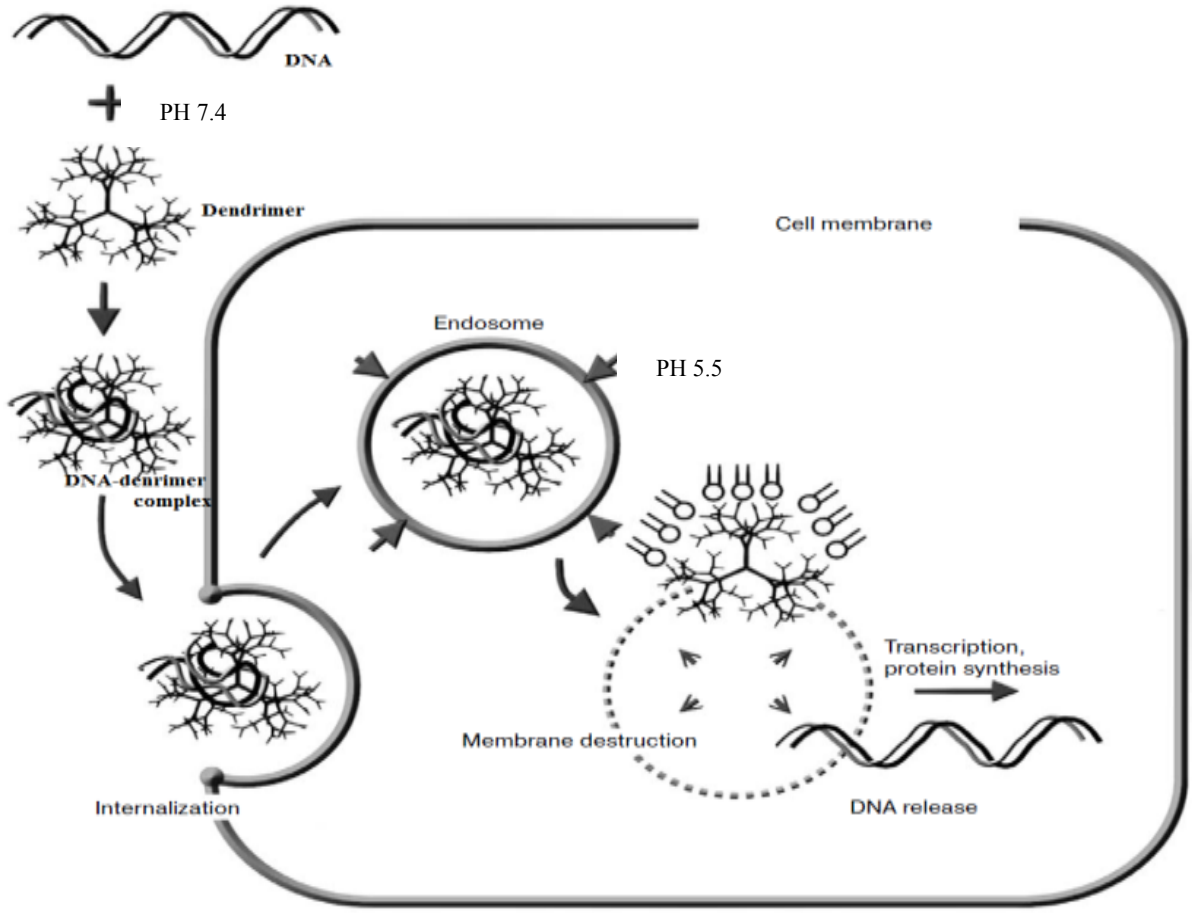

Figure1.12: Genetic material (DNA) transfer using dendrimers (Shcharbin et al., 2009).

Several studies proved the ability of using dendrimers as DNA vehicle; Haensler and Szoka were the first who reported in 1993 that plasmid DNA containing luciferase and β-galactosidase genes can be delivered into cells using PAMAM dendrimers. In 1996, Baker, et al. published results of investigation of efficiency of genetic material transfection using dendrimers with different cell lines. It was shown that protonated dendrimers in a wide range interact with negatively charged plasmid DNA, and the formed complex is stable under physiological conditions even in the presence of sodium dodecyl sulft (SDS).

1.4.1 Importance of charge inversion

In nature the cell membrane of the living cell carries the same net negative charge as DNA, which means if DNA is delivered into the cell as nacked-DNA it will not be able to penetrate the cell membrane and reach inside the cell because of the repulsion force between the cell membrane and DNA molecules. Using dendrimer as DNA vector will solve this problem: when a dendrimer interacts with DNA it forms a complex, if the dendrimer carries a positive charge sufficient to neutralize or invert the DNA charge

making the net charge of the resulting complex positive, this complex will overcome the negative charge of the cell membrane facilitating the penetration of DNA inside the cell. Computer simulation of complexes formed by charged dendrimers and oppositely charged linear molecules of DNA was first performed by Welch and Muthukumar (Welch et.al.;2000). They investigated the case when a dendrimer charge exceeded the charge of the chain and drew attention to the effect of overcharging on the complex.

1.4.2 Condensation of DNA by dendrimers

A compact conformation will appear due to the electrostatic interaction between LPE chain and dendrimer, which reduces the size of LPE chain because of its ability to wrap around the dendrimer, this resulting complex resemble the histone-DNA complex in the living cell. This conformation proved the protection for the LPE chain will increase the efficiency of using dendrimer as DNA vector.

1.4.3 Effect of pH on complex formation

pH value has a great effect on the size, structure and behavior of dendrimers, depending on the type of basic interior and terminal group of the dendrimer. If we take PAMAM dendrimers with interiors containing tertiary amines, at low pH ($pH \leq 4$) the electrostatic repulsion between the positively charged ammonia groups increase this will leads to extending the dendrimer conformations. Which means that the interior structure of dendrimer getting increasingly "hollow" as the generation number increases. At neutral pH, back-folding occurs which may be a consequence of hydrogen bonding between the uncharged tertiary amines in the interior and the positively charged surface amines. But at higher pH ($pH \geq 10$) the dendrimer contract as the charge of the molecule becomes neutral, acquiring a more spherical (globular) structure due to weak repulsive force, see figure 1.13.

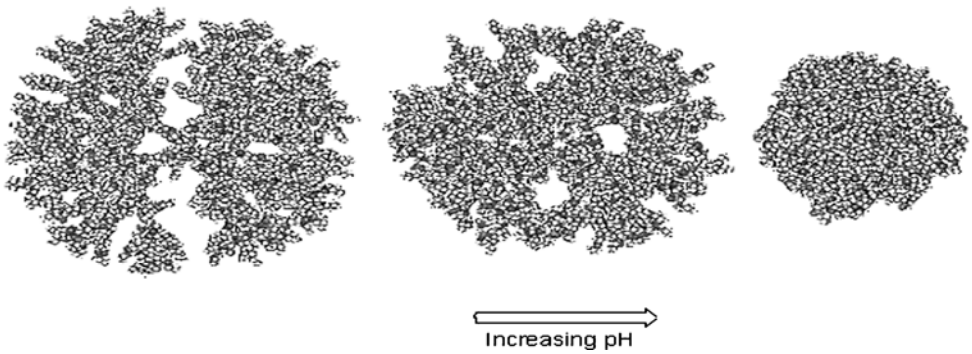

Figure 1.13: Three-dimensional structure of a G6-PAMAM dendrimer, under different pH. Calculation is based on molecular dynamics (Boas et al., 2006).

This effect also plays a big role in complex formation and conformation, Maiti and co-workers studied the effect of pH value on complex, they studied complexation between oligonucleotides (single strand DNA) and various generation of ethylenediamine (EDA) cored of PAMAM dendrimers through atomistic molecular dynamics simulations, they proved that at high pH no complex will form because the dendrimer is uncharged at this pH, at neutral pH strong electrostatics interaction between dendrimer and ss-DNA due to the protonation of all the primary amines. Lowering pH increase the wrapping of DNA on the dendrimer according to the generation of dendrimer. This result help understand the mechanism of controlling the DNA release inside the cell by controlling the pH value.

1.5 Statement of the Problem

Most of the previous studies, models and simulations deal with the dendrimer in the complex as a hard sphere (rigid , radius fixed , DNA can't penetrate the dendrimer), but the study of Maiti (Maiti et al.,2006), Qamhieh(Qamhieh, et al.,2009) and others showed that under some circumstances the linear polyelectrolyte chain (LPE) penetrates the dendrimer, which means that the dendrimer can be considered as a soft sphere (penetrable sphere) where the radius of the dendrimer can be changed, DNA and ions can penetrate the dendrimer, (see figure1.14).Qamhieh and co-worker (Qamhieh et al.,2011) developed a soft sphere model relying on a hard sphere model for Schiessel. They applied the new model to study the complexation of LPE chain with some generations of ammonia and ethylenediamine cored dendrimers. In our study we will extend the investigation on the complexation in 1:1 salt solution of the same LPE chains with ammonia and ethylenediamine cord dendrimers of some generations that haven't been investigated in other previous reaches, by applying the new penetrable sphere model. This will increase the clearance of the behavior of dendrimers/LPE chain complexes used in transferring the DNA into the cells in gene therapy.

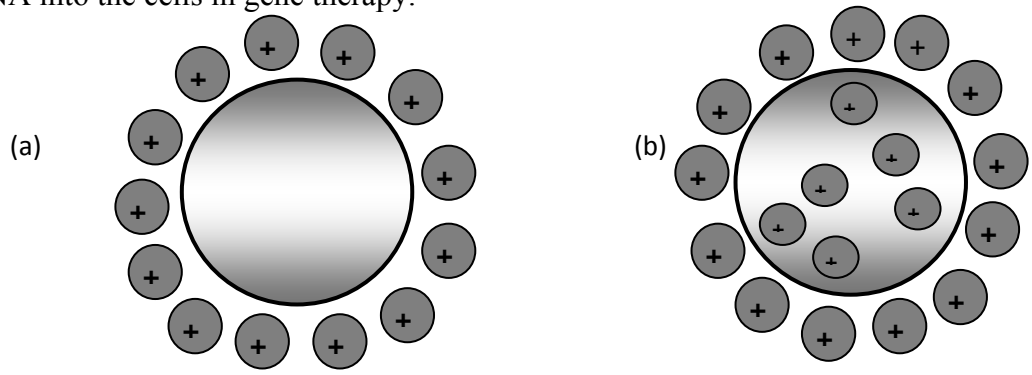

Figure 1.14: Schematic of (a) a hard-sphere and (b) a soft sphere.

Chapter Two

Method and Model

Chapter Two

Method and Model

2.1 Introduction

The method of study is mainly based on using a new model developed by Qamhieh (Qamhieh et al.,2009) in which some modifications has been made to a model that was developed by H. Schiessel(Schiessel et al.,2001), and entering all the variables into this model to study their effect on the complex taking into account that the dendrimer is regarded as soft sphere.

2.2 Analytical model of the system

Qamhieh and co-workers considered in the model the complexation between soft sphere(ion penetrable sphere) of radius R, charge Ze and LPE chain as semiflexible rod of radius r = 1 and contour length L, where $L>>R$. The charge of LPE chain per unit length is $-e / b$, where b the distance between the charge on the chain which is much smaller than Bjerrum length l_B according to the experimental conditions, the salt solution characterized by Bjerrum length $l_B = e^2/\varepsilon k_B T$ (ε: dielectric constant of the medium, T: absolute temperature) and Debye screening length is $\kappa^{-1} = (8c_s\pi l_B)^{-1/2}$($c_s$: Concentration of salt).

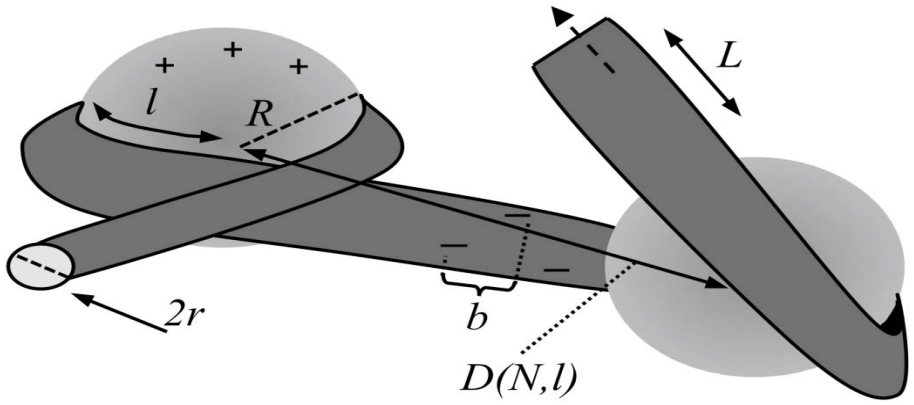

Figure 2.1**:** Proposed binding model between DNA of contour length L, radius r, and distance between negative charges b and G4 PAMAM dendrimers modeled as hard spheres of radius R. The DNA is shown to wrap around the dendrimer with the length of the wrapping part equal to l, and a distance between 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 Free energy calculation for a complexation between a LPE chain and a single dendrimer

The total free energy can be given similar to Schiessel model as:

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

Where l is the length of the part of DNA molecule wrapped around the sphere, and $(L - l$ is the length of the remaining chain.

The first term is the electrostatic charging free energy of spherical complex of charge $Z(l)e$ where the sphere is considered as a soft sphere (ion penetrable sphere):

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

The total charge of the complex can be given by, $Z(l) = Z - l/b$, where the complex represents the sphere and the corresponding wrapped chain.

The second term of the total entropic electrostatic free energy of the remaining chain. (***L-l***) can be kept similar to Schiessel model and 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 Ω (a) is the entropic cost to 'confine' counterions close to the chain.

The third term is the resulting free energy between the complex (as soft sphere) and the unwrapped segment of the LPE chain is given by:

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

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 Scheissel's model:

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

2.4 Free energy calculation for a complexation between a LPE chain and a multiple dendrimers

The total free energy for a system consisting of one LPE chain and *N* number of spheres is given by

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

Where $F(l)$is the total free energy of the PE chain - sphere complex, F_{int} is the repulsive electrostatic interaction between *N* ion-penetrable spheres that decorating the polymer chain.

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, it is given as:

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

Where $D(N,l)$ is the center-to-center distance between two complexes next to each other which equals to $(L-Nl+2NR)/N$, is small at very low ionic strength, This interaction energy is worth nothing as this term will be small if the charge of complex is close to neutral.

Finally the total free energy of the system is represented by the following:

$$\frac{F(N,l)}{k_BT} = \frac{3NZ(l)l_BA}{4\pi(\kappa R)^2}\left[\frac{Z(l)}{2}\frac{e^{-\kappa R}}{R} + \frac{1}{b}\cdot\int_R^{L-Nl}\frac{e^{-\kappa r}}{r}dr + \frac{3Z(l)A}{2(\kappa R)^2}\sum_{i=1}^{N-1}\left(\frac{N-i}{i}\right)\frac{e^{-\kappa D}}{D}\right] + \frac{1}{b}\cdot\Omega(a)\cdot(1-\xi^{-1})\cdot(L-Nl) + \frac{Nl_p}{2R^2}l. \qquad (2.7)$$

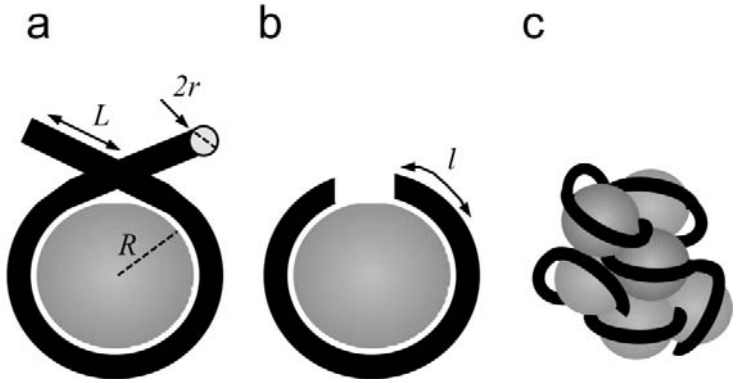

Figure 2.2: (a) a piece of a DNA molecule is shown to wrap around one dendrimer. The DNA segments linking to the next dendrimer in an aggregate. (b) a dendrimer/DNA complex consisting only of one

dendrimer and the DNA segment actually wrapping the dendrimer, *l*. (c) the dendrimer/DNA aggregate consisting of the entire DNA molecule and a multiple of dendrimers (Qamhieh et al., in process).

The types of dendrimers that has been used in our study is polyamidoamine (PAMAM) with different cored ammonia cored (NH_3) and with ethylenediamine cored(EDA), of different generations (G) and each generation has its own specifications that shown in table (2.1). These variables will be applied in the new developed model to study the dendrimer – DNA complex.

Table 2.1: Charge and radius of PAMAM generations as provided from the manufacturer, Dendritech.

Generation (G)	Ammonia cored dendrimer		Ethylenediamine cored dendrimer	
	Charge (Z)	Radius(nm) (R)	Charge (Z)	Radius(nm) (R)
1	6	1.58	8	1.1
2	12	2.2	16	1.45
3	24	3.2	32	1.8
4	48	4	64	2.25
5	96	5.3	128	2.7
6	192	6.7	256	3.35
7	384	8	512	----
8	768	----	1024	4.85

The second step is to compare the result that has been obtained from our investigation with previous studies done on the same LPE chain to get more information that could help understand and analyze the dendrimer-DNA complex and the mechanism of releasing DNA from dendrimer inside the cell, which increases the possibility of using dendrimer in gene therapy and other applications.

Chapter Three

Results and Discussions

Chapter Three

Results and Discussions

In this study the optimization method for finding the minimum of the total free energy for a system of soft spheres that interact with LPE chain of contour length L is used by the essential tool of mathematics and modeling Maple 12, we also use the Origin lab program of version 8 for graphing and analyzing some of the theoretical results.

3.1 Single PAMAM dendrimer – LPE chain complex

Through our study the optimal wrapping length (l_{opt}) of LPE chain which has been wrapped around dendrimer has been found by taking the first derivative of the total free energy equation for penetrable sphere model (Eq.(2.7)) with respect to the wrapping l_{opt}, then equating it to zero (i.e., the total free energy equation has to be minimized).

The complexation of LPE chain and single dendrimer in a 1:1 salt solution of NaBr has been investigated through this study by the penetrable sphere model. Many factors control the formation of this complex, such as the Bjerrum length in the medium, the dendrimer charge, the salt concentration in case of 1:1 salt solution, the chain length and the effect of LPE chain rigidity(l_p) on the complex formation that has been studied in this investigation.

3.1.1 Effect of Bjerrum length on PAMAM dendrimer – LPE chain complex conformation:

The complexation of flexible LPE chain of persistence length l_p= 3nm was chosen to bear 47 negatively charged monomers (-47e) in the system of monovalent chain (single strand) with charge spacing b = 0.67nm and 94 negatively charged monomers (-94e) in the system of divalent chain (double strands) with charge spacing b = 0.335nm, in both systems the LPE chain length L ≈32nm, and ammonia cored PAMAM dendrimer of generation three (G3) holding 24 positively charge (Z = 24)at physiological (neutral) pH conditions, with radius of 3.2nm (R = 3.2nm). Both systems have been studied by the penetrable sphere model at 1:1 salt concentration corresponds to Debye screening length (DSL) of 6 nm. The fraction of the condensed monomers of the LPE chain of charge –e on the PAMAM dendrimer of generation 3 (l_{opt}) and the total charge of the complex is analytically studied for different Bjerrum lengths which measures the strength of electrostatic attraction

between LPE chain and dendrimer complexes. The net charge of the complex can be calculated in terms of $Z_{compl} = Z_{ded} - Z_{cond}$, where Z_{dend} is the total charge of the positively charged dendrimer in the case of ammonia cored PAMAM dendrimer of generation three (G3), and Z_{cond} is the total charge of the chain that is condensed on the dendrimer, it can be written in terms of l_{opt} / b.

As it is shown in Figure 3.1a,b for both monovalent and divalent chain, for small values of Bjerrum length , for weak electrostatic interaction , there is small fraction of the condensed monomers on dendrimer which means that the tail is the dominant, By increasing the ratio between the Bjerrum length and monomers spacer *b*, the chain tails disappear very quickly. The calculated maximum fraction of the condensed monomers of the LPE chain by our study was found to reach to 0.97 and 0.95 for monovalent and divalent chains respectively, these results are compared with molecular dynamic simulation (Lyulin et al;2008) and penetrable sphere model (Qamhieh et al) for ammonia cored dendrimer of generation G4 at the same condition, both results agree with our calculation and shows a saturation in the condensed monomers at the same value of the ratio between the Bjerrum length and monomers spacer, i.e., at $l_B \geq 3.25b$. In both monovalent and divalent DNA strands systems we noted that G4 is effected by Bjerrum length change more than G3 due to the difference in charge value. But we noted that G3 ammonia is less effected in the change in Bjerrum length than G4 ammonia cored due to the difference in charge and radius value which influence the electrostatic interaction.

We extended our study to cover the effect of Bjerrum length on the complexation of ethylenediamine cored PAMAM dendrimer of different generations (G2, G3, G4) with same flexible chain (L =32nm, l_p =3nm, DSL =6nm). Figure 3.2(a,b), shows that the complex at weak electrostatic interactions, $l_B < 1b$,there is small fraction of the condensed monomers on dendrimer which means that the tail is the dominant, and this tails are getting shorter very quickly as Bjerrum length is increased, and are about to disappear for $l_B \approx b$, and a saturation in the condensed chain is achieved in both cases on monovalent and divalent chains in each PAMAM dendrimer for different generations. From the figure 3.2 it obvious that the effect of Bjerrum length varied from one generation to the other of the same cored according to the difference in charge and radius value. These results agree with what has been concluded by MD simulation done by Lyulin et al. and the calculations on penetrable sphere model done by Qamhieh et al, on G4 ammonia cored dendrimer and has

the same behavior as l_B change. Our result are conflict with the worked done by Schiessel et al, on hard sphere model for generation G4 ammonia cored PAMAM dendrimer with same LPE chain as the previous study. It shows that for small values of Bjerrum length we get negative values of LPE wrapping length for both monovalent and divalent chain which is not a reasonable result, also the fraction of the condensed monomers in the case of divalent chain exceeds the unity, so it can't be compared to our conclusion .

Figure 3.3, shows the effect of Bjerrum length on G4 dendrimers of ammonia cored (Qamhieh,et.al) and ethylenediamine cored, we note that both types have the same behavior or pattern but with minor variation due to the difference in charge and radius value which causes difference in charge distribution on the dendrimere , that approve the agreement of our result with the previous work of Qamhieh.

Figure 3.4 a, shows the normalized effective charge of the complex Z_{compl}/Z_{dend} as a function of Bjerrum length l_B, It is obvious from the figure that the divalent chain is more effective in neutralizing charge dendrimer than the monovalent chain, where the normalized charge of the complex is reduced from 0.79 to -0.93 with monovalent chain and from -0.44 to-2.74 with divalent chain when the Bjerrum length increased to 8b, which means that the charge of the dendrimer is inverted to negative value, as the divalent chain contains charges more than the dendrimer. This results agree with MD simulation done by Lyulin et al, and with penetrable sphere model calculation done by Qamhieh et al, see figure3.4b .Also the effect of Bjerrum length on the normalized charge of the complex has been studied by the hard sphere model developed by Schiessel, from his study on both system monovalent and divalent, he found that the ratio between the complex charge and dendrimer charge was large at small value of Bjerrum length and this disagreed with the result that was obtained by the soft sphere model.

We extended our study to cover the effect of Bjerrum length on normalized charge of the complexation of ethylenediamine cored PAMAM dendrimer of different generations with same semi flexible chain. Figure 3.5 shows that the divalent chain is more effective in neutralizing charge dendrimer than the monovalent chain, and as the Bjerrum length is increased the normalized charge of the complex will decrease.

Figure 3.6, shows the charge of the counterions condensed on G3-LPE chain complex normalized to dendrimer charge as a function of Bjerrum length, as it is shown the average fraction increases with the increasing of Bjerrum length and this fraction of the condensed

counterions on the G3-LPE complex in the case of the complexation of dendrimer with monovalent chain is larger than that of the complexation of dendrimer with divalent chain and this can be explained by the fact that the divalent chain is more effective in neutralization dendrimer charge.

(a) (b)

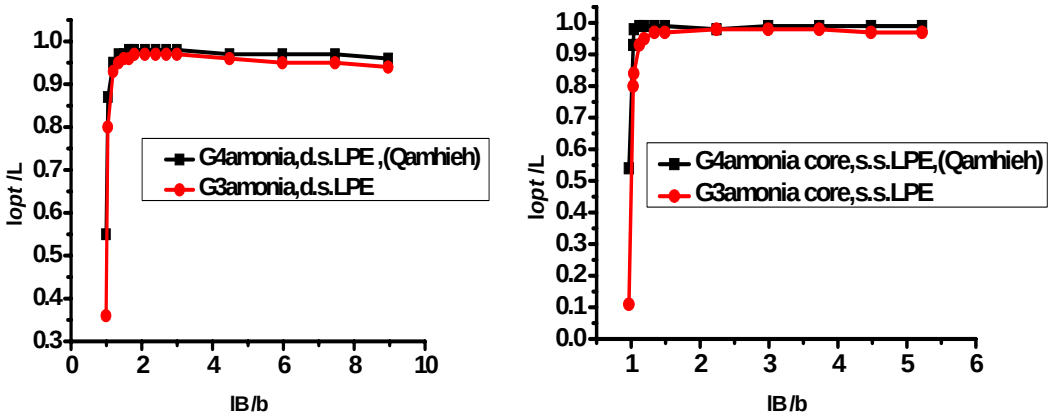

Figure3.1 Fraction of optimal LPE chain condensed on a charged dendrimer of G3and G4 ammonia cored with (a)divalent LPE chain (b) monovalent LPE chain as a function of Bjerrum length l_B. The dendrimer is complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

(a) (b)

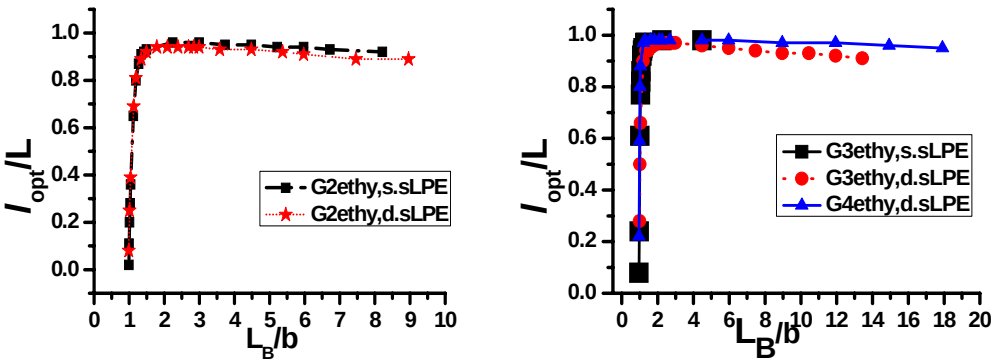

Figure 3.2: Fraction of optimal LPE chain condensed on a charged dendrimer of different generation G ethylenediamine cored as a function of Bjerrum length l_B, (a) G2 ethylenediamine cored, (b) G3 and G4 ethylenediamine cored . The dendrimer modeled complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

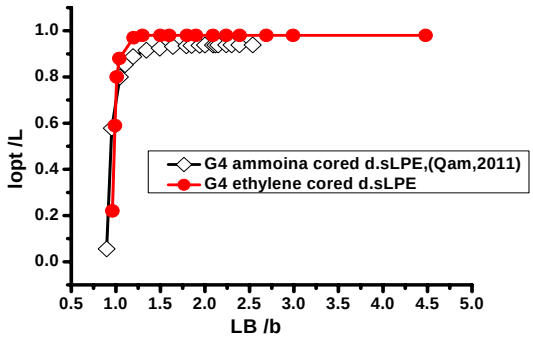

Figure3.3: Fraction of optimal LPE chain condensed on a charged dendrimer of G4 of both ammonia and ethylene cored dendrimer with divalent LPE chain as a function of Bjerrum length l_B. The dendrimer modeled complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

(a) (b)

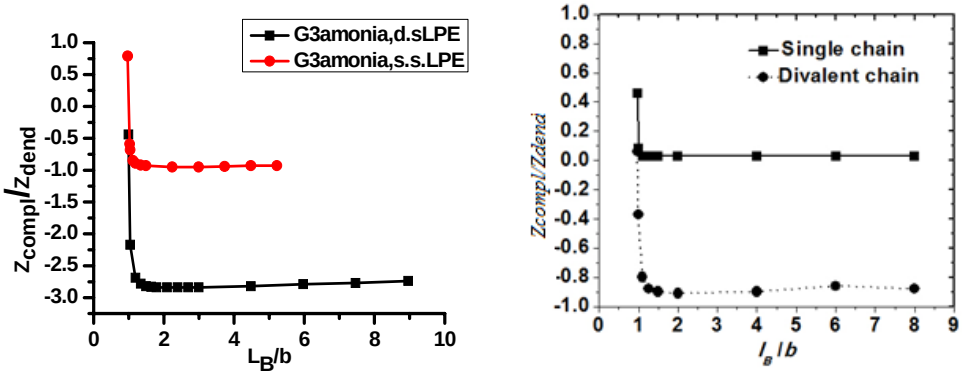

Figure 3.4: (a)The normalized charge of the complex as a function of Bjerrum length l_B. The dendrimer G3 ammonia modeled as a sphere of radius R=3.2nm and charge Z= 24, complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm, (b)the normalized charge of the complex as a function of Bjerrum length l_B, for dendrimer G4 ammonia modeled as a sphere of radius R=4and charge Z= 48 (Qamhieh et al).

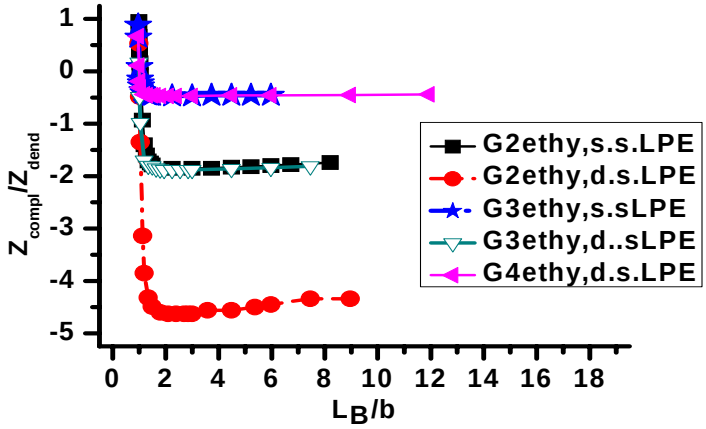

Figure3.5: The normalized charge of the complex as a function of Bjerrum length l_B. Different generation of ethylene cored dendrimer complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

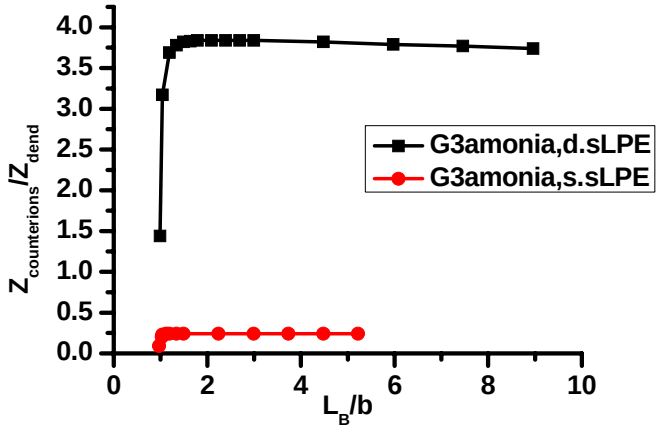

Figure3.6: Simulation results for $Z_{counterions}/Z_{dend}$ condensed on dendrimer G3-LPEchain complex as a function of Bjerrum length.. The dendrimer G3 ammonia modeled as a sphere of radius R=3.2nm and charge Z= 24, complexed with an oppositely charged flexible LPE chain of persistence length l_p=3nm at 1:1 salt concentration corresponding to Debye screening length of 6nm.

3.1.2 Effect of salt concentration on PAMAM dendrimer – LPE chain complex conformation:

The complexation of flexible LPE chain of 33nm length chain with oppositely charged ethylenediamine cored PAMAM dendrimer G3 of radius R=1.8nm and charge of Z_{dend}=32, has been studied at different concentration of 1:1 salt solution at room temperature, and at two values of Bjerrum length l_B,0.7 and 0.71nm. The charge spacing of the chain is b=0.7nm, and the persistence length l_p=3nm. Figure3.8 shows the fraction of chain length

condensed on the dendrimer as a function of reciprocal Debye length k, which is proportional to salt concentration, from which we can conclude that, as the concentration of salt is increased the optimal length is increased which means that the LPE chain become more wrapped around dendrimer, but 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 measured by Bjerrum length l_B. As it is shown in the figure3.7, the effect of l_B on optimal length is insignificant at high salt concentration, but at very small salt concentration the optimal length is very sensitive to the strength of the electrostatic interaction, which means that a small increase in Bjerrum length, will result in a high increase in the optimal length. Eq. (2.7) for the total free energy includes two terms that tend the LPE chain to resist wrapping around dendrimer namely, the mechanical bending free energy and the electrostatic repulsive free energy between the chain charges, the latter loses its importance as the salt concentration is increased due to the screening effect, these repulsions are balanced by the electrostatic attraction between dendrimer and LPE chain, which favors the bending of LPE chain in order to wrap around dendrimer. Overcharging of the dendrimer is observed to be larger at higher salt concentration. Our results are in good agreement with what has been calculated by Qamhieh et al on ammonia cored PAMAM dendrimer G3, see figure 3. 8, also agree with the theoretical studies (Nguyen and Shklovskii, 2001; Boroudjerdi et al.,2011) and computer simulation studies (Lyulin et al., 2005; Larin et al., 2009; 2010) . Also from the 3.7 we noted that the influence by the salt concentration varied differently from G3 ammonia cored and G3 ethylenediamine cored at the same value of Bjerrum length which clear the effect of charge and radius effect as the salt concentration change.

To support our result further work on the salt concentration effect on PAMAM dendrimer – LPE complex, was done on system of ammonia cored PAMAM dendrimer of G2, G3and G4 of radius R=2.2nm, R= 3.2nm and 4nm and charge of Z_{dend}= 12, 24and 48, has been studied at different concentration of 1:1 salt solution at room temperature, and at two values of Bjerrum length l_B,0.7 and 0.71nm, with LPE chain of length L=1472.5nm, persistence length l_p=50nm and spacer b=0.17nm . Results appear to agree with the previous studies, and ensure that as the salt concentration increase the optimal length is increased at small value of k, bout at higher salt concentration the optimal length is almost fixed due to a finite length LPE chain. And the effect of Bjerrum length on the optimal

length is obvious at small salt concentration but at higher concentration it becomes insignificant as explained before, see Table 3.1 and figure3.8.

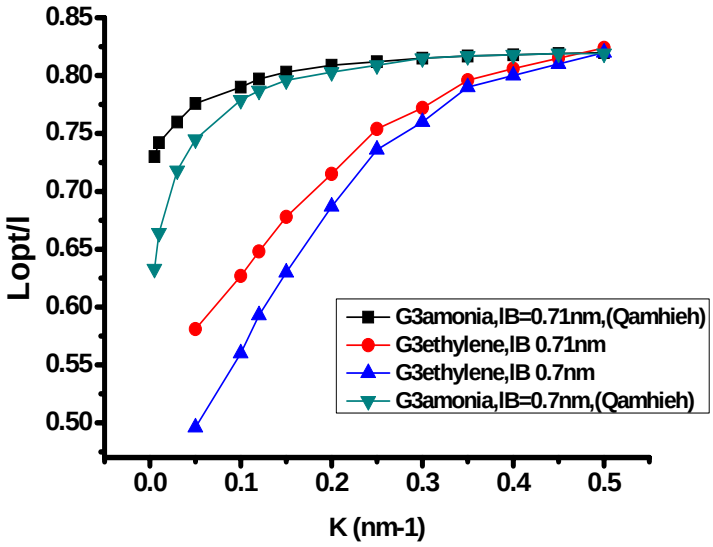

Figure 3.7: Fraction of condensed monomers of LPE chain on PAMAM dendrimer G3 for both ammonia and ethylenediamine cored as a function of salt concentration 1:1 salt solution, the length of LPE chain equal to L=33nm with spacer of 0.7nm,of persistence length equals to 3nm.

Table 3.1: Analytical model results for the interaction between G_x dendrimer and LPE chain, the dendrimer is considered to be a ion-penetrable sphere of radius R.

C_s (mM)	L_{opt}					
	G2(l_B=0.71nm)	G2(l_B=0.7nm)	G3(l_B=0.71nm)	G3(l_B=0.7nm)	G4(l_B=0.71nm)	G4(l_B=0.7nm)
5	187	188	499	501	873	876
10	213	214	609	611	1053.5	1056.5
30	292	293	906	909	1255.8	1257
50	367	368	1093	1094.6	1284.9	1285.1
100	550	551	1231	1231.7	1282.7	1282.7
120	639	639	1246.7	1247.2	1276.6	1276.7
150	760	760	1255.3	1255.5	1268.9	1268.9
200	916	916	1257.6	1257.9	1260.2	1259.8
250	1063	1062.5	1255.6	1255.8	1253	1253.1
300	1128	1127.5	1253.4	1253.5	1249.7	1249.9
350	1183	1182.7	1250.2	1250.2	1246.9	1246.9
400	1196	1196	1249.2	1249.2	1246.2	1245.8
450	1212	1211.7	1247.7	1247.7	1245.4	1245.3
500	1220	1219.8	1247	1246.9	1245	1244.7

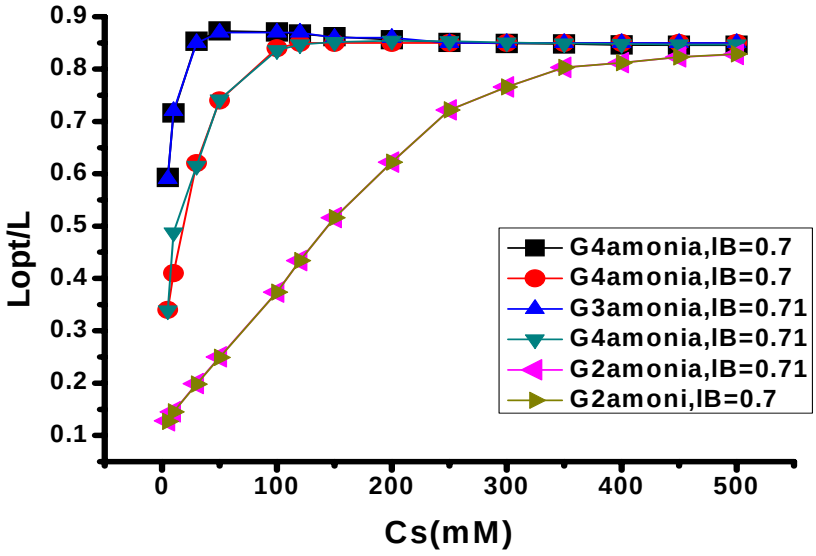

Figure 3.8: The fraction of the condensed monomers of LPE chain as a function of 1:1 salt concentration for a system of one dendrimer of G_x and an semiflexible LPE chain of persistence length l_p=50nm representing DNA of 4331bp (L=1472.5nm).

3.1.3 Effect of chain stiffness (Persistence length) on PAMAM dendrimer – LPE chain complex conformation:

The complexation of LPE chain of 1472.5nm length, represent plasmid DNA, with oppositely charged ethylenediamine cored PAMAM dendrimer G4 of radius R=2.25nm and charge Z_{dend}= 64, has been studied using the penetrable sphere model, at different degrees of chain rigidity measured in terms of persistence length(l_p). Two values of Bjerrum length are considered, l_B= 0.71nm represent the complex in water solution, l_B= 6.7nm represents the complex in the core of the cell membrane. The complexation has been studied with single strand LPE chain with charge spacing b = 0.34nm and divalent strand LPE chain with charge spacing b= 0.17nm.

Figure 3.9 a, b, shows the optimal chain length in all cases. From the figure, we can approve that the optimal wrapping length around the dendrimer decreases in all cases as the rigidity of the chain increases, this effect is more obvious at small Bjerrum length in water solution, comparing to the system with large Bjerrum length in the core of the cell where its effect is insignificant, as the electrostatic interaction is strong. These results are in good agreement with the calculation of Qamhieh et al, that worked on the same LPE

chain but with different type of PAMAM dendrimer, ammonia cored G4 dendrimer. It is obvious that the effect of chain stiffness varied from ammonia cored to ethylenediamine cored dendrimers according to the difference in charge and radius value.

We extended our study by take another PAMAM dendrimer, G3 ethylenediamine cored PAMAM with of radius R=1.8nm and charge Z_{dend}= 32, to study the effect of radius and charge decrease on the system, we took the same LPE chain. Figure 3.11, shows that the decreased in optimal length round the dendrimer is more rapid and more significant with smaller sphere radius R= 1.8nm than R=2.25nm. And in case of divalent LPE chain the decrease is most rapid comparing to the other cases, and the length of the wrapping length is the largest. We concluded that the wrapping length is decreasing significantly as Bjerrum length is increased , and decreasing even more when the radius of the dendrimer is decreased , as the bending energy is increased by increasing radius of the sphere.

(a) (b)

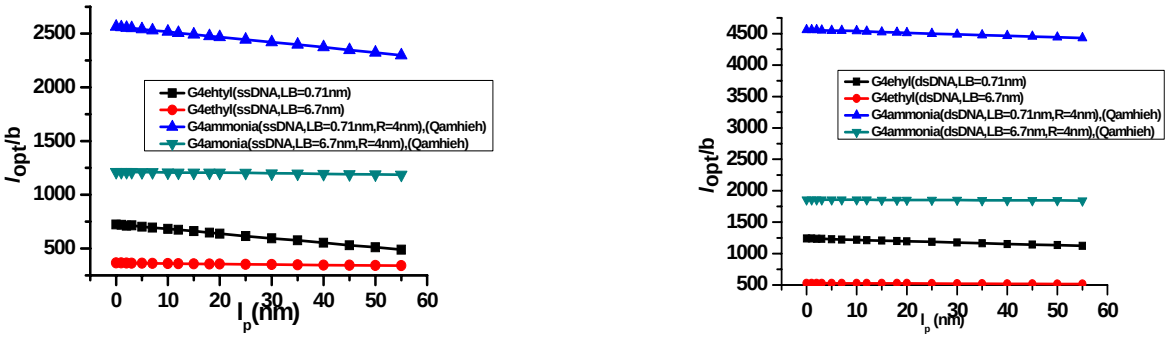

Figure 3.9: (a) The effect of persistence length l_p on the number of the condensed monomers of LPE chain on dendrimer of G4 both ammonia and ethylenediamine cored dendrimer, on a system of single strand DNA with spacer of 0.34nm, (b) The effect of persistence length l_p on the number of the condensed monomers of LPE chain on dendrimer of G4 both ammonia and ethylenediamine cored dendrimer, on a system of single strand DNA with spacer of 0.17nm.

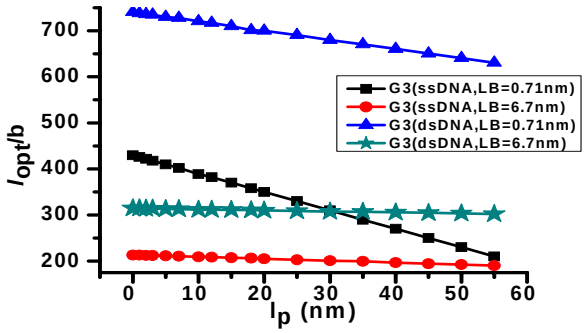

Figure 3.10: The effect of persistence length l_p on the number of the condensed monomers of LPE chain on dendrimer of G3 ethylenediamine cored dendrimer, on a system of single strand DNA with spacer of 0.34nm and a system of double strand with spacer of 0.17nm.

3.1.4 Effect of chain length on PAMAM dendrimer – LPE chain complex conformation:

The complexation between flexible LPE chain and an oppositely charged sphere has been studied at variant chain length L. The sphere modeled as PAMAM dendrimer G3 ethylenediamine cored of charge Z = 32 and radius R= 1.8nm and LPE chain of persistence length l_p = 3nm, at1:1 salt concentration corresponds to Debye screening length 6nm. Figure 3.11a shows the theoretical prediction of the condensed monomers of LPE on dendrimer by the penetrable sphere model for different length of LPE chain. Upon increasing of the chain length the number of the condensed monomers increase linearly until we reach a chain length which is critical and the number of the condensed monomers at this point is maximum, the overcharging of dendrimer is maximum. In general the decrease in the condensed monomers of LPE chain is attributed to the increasing in the electrostatic repulsive free energy between chain monomers which is larger at maximum overcharging of the dendrimer.

Figure3.11b, shows that as the length of LPE chain increase the number of turns rounded the sphere is increased linearly until it reach a chain length maximum point where the overcharging of dendrimer is maximum, and this agree with the result got by Qamhieh and coworker (Qamhieh., et al) for G3 ethylenediamine cored dendrimer.

(a) **(b)**

Figure 3.11: (a) The number of condensed monomers as a function of chain length. The complex composed of flexible LPE chain of persistence length lp=3nm at 1:1 salt concentration corresponds to Debye screening length of 6 nm with G3 dendrimer (for two type of cored ammonia and ethylenediamine cored),(b) the number of turns warp around dendrimer as a function of chain length, for the same pervious system.

3.1.5 Effect of PAMAM dendrimer charge on PAMAM dendrimer – LPE chain complex conformation:

To cover the effect of dendrimer charge on the complex formation, we took three different generations and types of dendrimer to compare the change in behavior of each on when the charge of dendrimer change which resemble the change in pH value because it found that as pH value change the dendrimer charge change. A system composed of LPE chain with length of L= 50 nm, persistence length l_p = 50nm, and charge spacing of b= 0.17nm, at salt concentration 100mM of 1:1 salt solution which corresponds to 0.96nm of Debye screening length and at 0.71nm of Bjerrum length have been studied at different dendrimer charge using penetrable sphere model with PAMAM dendrimer of generation G5 ehyldiamin cored, G4 ehyldiamin cored and G4 ammonia cored dendrimer, with radius R= 2.7nm, 2.25nm and 4nm respectively.

Figure 3.12a, shows that the optimal wrapping length around the dendrimer is increased as the dendrimer charge are increased, and it is obvious by comparing G4 ehyldiamin dendrimer(R=2.25nm) with G4 ammonia dendrimer(R=4nm) that as the radius of dendrimer increased the effect of charge chain reduced, also we noted the same effect with a different dendrimer G5. From figure 3.12b, we can conclude that the number of turns rounded the sphere is increased linearly up to fixed value in each case as the dendrimer charge is increased, which is equivalent to decreasing pH. Figure3.13 shows that the total

charge of the complex(Z_{compl})is increased linearly as the charge of dendrimer increased and at higher charge of dendrimer it will start to reversed. Comparing this results with a previous work on penetrable sphere done by Qamhieh, et al but with a different type of dendrimer, and the work of Arcesi et al at (Arcesi et al at.,2007) hard sphere model, and with experimental results of E.Le Cam et al, all agree that as the charge of dendrimer increases optimal warping length and the number of turns around dendrimer increases.

Figure3.14, shows the effect of dendrimer charge on the overcharging degree $(l_{opt} - l_{iso})/l_{ios}$, where$l_{ios}$ is the isoelectric length of the chain, as it obvious from the figure that overcharging degree is decreased when the charge of the dendrimer is increased, in all dendrimer generations, and this agree with the previous result of Qamhieh et al.

(a) (b)

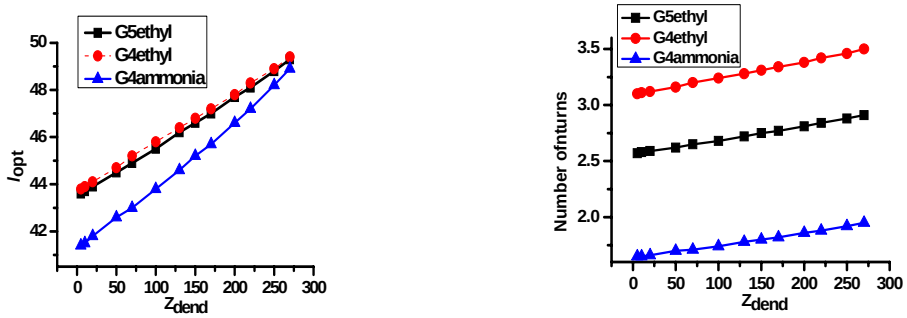

Figure3.12: Effect of dendrimer charge on (a) optimal warping length and (b)number of turns on the dendrimer around LPE chain, for a system of positively charged dendrimers each of charge $Z_{dend}$for three type of dendrimers and an appositely charged semiflexible LPE chain of length L =50nm,at salt concentration 100nM corresponds to 0.96nm of Debye screening length.

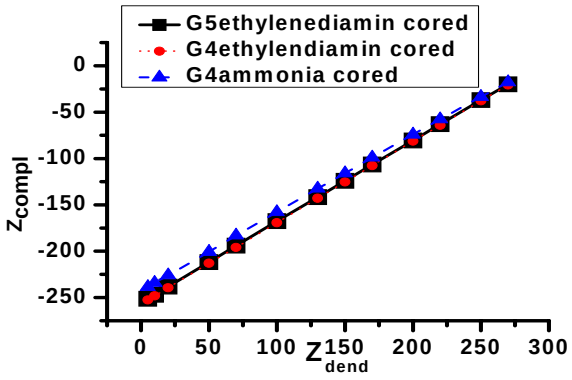

Figure 3.13: Effect of dendrimer charge on the total charge of the complex, for a system of positively charged dendrimers each of charge $Z_{dend}$for three type of dendrimers and an appositely charged semiflexible LPE chain of length L =50nm,at salt concentration 100nM corresponds to 0.96nm of Debye screening length.

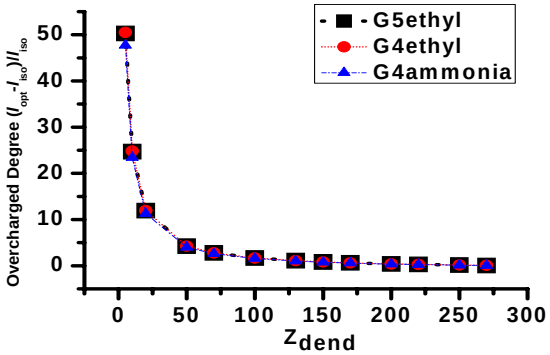

Figure 3.14: Effect of dendrimer charge on the overcharge degree(l_{opt}-l_{iso})/l_{iso}, for a system of positively charged dendrimers each of charge $Z_{dend}$for three type of dendrimers and an appositely charged semiflexible LPE chain of length L =50nm,at salt concentration 100nM corresponds to 0.96nm of Debye screening length.

3.2 System of a two PAMAM dendrimers-LPE chain complexes

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

Dendrimer has been considered as an ion-penetrable sphere as it was proved by Maiti and Bagchi (Maiti and Bagchi.,2006). The charge was found on the surface and was distributed inside the dendrimer. Also it was proved that dendrimer was a soft and highly flexible sphere (Rosenfeldt et al., 2002; likos et al.,2002),which means that the size and structure of dendrimer change when LPE chain wrap around it is due to electrostatic interaction between the opposite charge. This leads to the reduction on the radius of dendrimer ($R=xR_0$) according to Qamhieh and coworkers study(Qamhieh et al., 2009), and this will be taken into account in this part of the study.

The composition of aggregates formed between DNA(2000 base pairs(bp)) and two PAMAM dendrimer of G4 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 3nmof Debye screening length and 0.71nm of Bjerrum length. DNA is modeled as semiflexible rod of radius r =1nm and length L=680nm, the axial spacing between DNA charges is b =0.17nm, and persistence length l_p is 50nm, which is large comparing to the dendrimers radius.

Tables 3.2 and 3.3 show the effect of the reduction of the dendrimer size on the optimal wrapping length of LPE chain around dendrimer, the total charge of the dendrimer –LPE aggregate, and other structural properties of the aggregates. The dendrimers are considered to be a penetrable sphere with dendrimer-dendrimer spacing D(N,*l*)=(L-N*l*+2NR)/N.

From figures 3.15and 3.16, we can note that as the reduction of dendrimer radius increased the optimal wrapping length is decreased as the number of LPE chain wrapping around the dendrimers is increased, also the linker length D' is increased and the total charged of the complex is increased. This result agree with the result of Qamhieh and coworker for different generations (G2,G4,G6,G8) of ethylenediamine cored dendrimer (Qamhieh et al .,2009; Qamhieh et al).

Table3.2: Analytical model results for the interaction between two G4 ethylenediamine cored dendrimers and LPE chain (2000bps), L=680nm, Z_{dend}=64 and $L_{iso}$is found to be 10.88.

X ($R=XR_0$)	l_{opt}(nm)	Diff (nm)	$Z^*_{complex}$	Z*/Z	D(N,l) (nm)	D'=D2R (nm)	(D'+Diff)N/L	$l_{opt}/2\pi R$
1.0	16.4	5.52	-32.47	-0.51	7.53	3.03	0.44	1.16
0.9	16.2	5.32	-31.29	-0.49	7.29	3.23	0.44	1.27
0.8	15.8	4.92	-28.94	-0.45	7.23	3.63	0.44	1.40
0.7	15.5	4.62	-27.18	-0.42	7.09	3.93	0.44	1.56
0.6	15.1	4.22	-24.82	-0.39	7.03	4.33	0.44	1.78
0.5	14.6	3.72	-21.88	-0.34	7.09	4.83	0.44	2.06
0.4	13.5	2.62	-15.41	-0.24	7.73	5.93	0.44	2.39

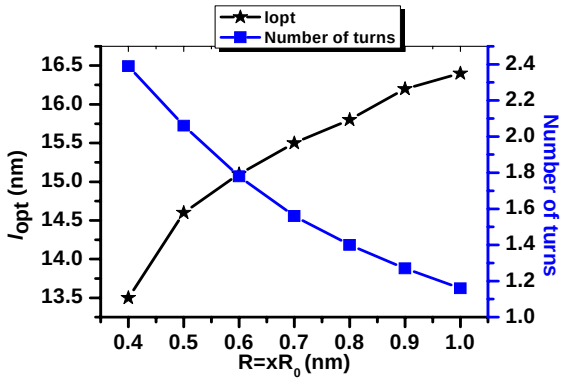

Figure 3.15: Effect of radius reduction on optimal wrapping length of LPE chain around dendrimer and on numbers of turns around dendrimer. For a system composed of LPE chain of length equal 680nm and two PAMAM dendrimer of G4 ethylenediamine cored of a charge Z_{dend}= 64.

Table3.3: Analytical model results for the interaction between two G4 ammonia cored dendrimers and LPE chain (2000bps), L=680nm, Z_{dend}=48 and $L_{iso}$is found to be 8.16.

X ($R=XR_0$)	l_{opt}(nm)	Diff (nm)	$Z^*_{complex}$	Z*/Z	D(N,l) (nm)	D'=D2R (nm)	(D'+Diff)N/L	$l_{opt}/2\pi R$
1.0	17.4	9.24	-54.35	-1.13	10.03	2.03	0.58	0.69
0.9	17.1	8.94	-52.59	-1.10	9.53	2.33	0.58	0.76
0.8	16.7	8.54	-50.24	-1.05	9.13	2.73	0.58	0.83
0.7	16.2	8.04	-47.29	-0.99	8.83	3.23	0.58	0.92
0.6	15.7	7.54	-44.35	-0.92	8.53	3.73	0.58	1.04
0.5	15.2	7.04	-41.41	-0.86	8.23	4.23	0.58	1.21
0.4	14.5	6.34	-37.29	-0.78	8.13	4.93	0.58	1.44

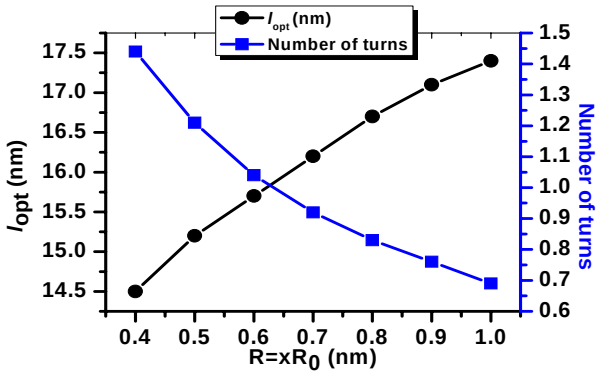

Figure 3.16: Effect of radius reduction on optimal wrapping length of LPE chain around dendrimer and on numbers of turns around dendrimer. For a system composed of LPE chain of length equal 680nm and two PAMAM dendrimer of G4 ammonia cored of a charge Z_{dend}=48.

3.2.2 Linker formation between complexes:

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

Figure 3.17 shows the effect of LPE chain length on linker formation between different generations of dendrimers G3 and G4 for both ammonia and ethylenediamine cored. The total length of LPE chain is divided into two parts, the optimal wrapping length and the linker, it is obvious from the figure that as the length of the chain increased the optimal wrapping length increases and also the linker increases.

Figure 3.18 shows the effect of Bjerrum length on a complexation of dendrimers of G3 (both ammonia and ethylenediamine cored) with flexible LPE chain of length 80nm. From the figure we can see that the optimal wrapping length increases as ratio of L_B/b increases but on the other hand linker decreases as this ratio increased. This means that the number of condensed monomers of the chain on the dendrimer increased which leads to the reduction of the linker between the complex, as well as to the reduction of positive charge of the complex, as a result the repulsive electrostatic interaction between complexes decreases.

Table 3.4 shows the different between G3 ethylenediamine and G3ammonia cored, and shows the effect of Bjerrum length in each one, we notes that as LB/b ratio increases the optimal wrapping length increases in both cases and the difference between the two types has become insignificant as the ratio increases.

Table 3.5 present the effect of pH value on the linker formed between 2G6-LPE chain complex. We note that at acidic condition optimal wrapping length decreases and linker increases and the opposite happens at basic condition.

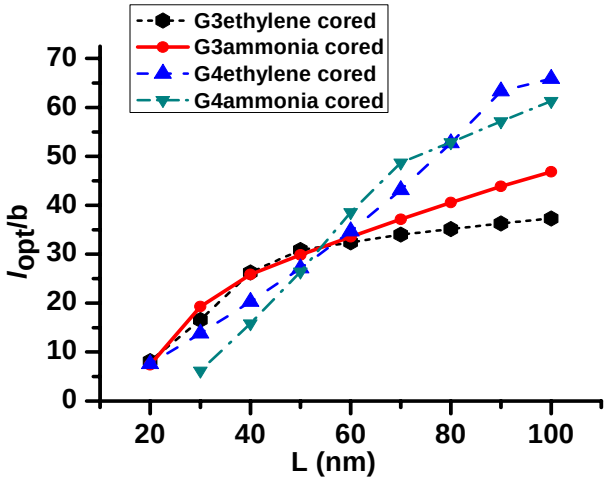

Figure 3.17: Effect of LPE chain length on linker formation and optimal wrapping length, for a system composed of 2 Gx dendrimer and flexible LPE chain of persistence length 3nm and spacer of 0.7nm, 100nm of Debye screening length.

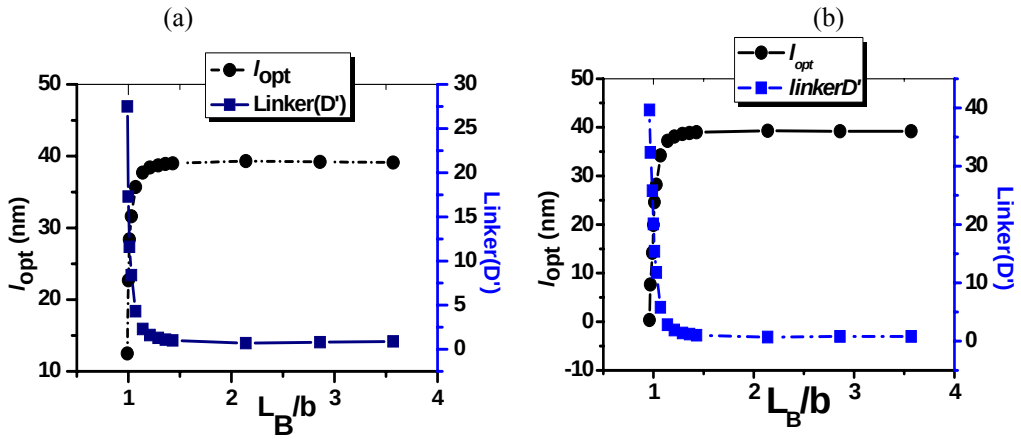

Figure 3.18: Linker formed between 2G3complexes with flexible LPE chain as a function of Bjerrum length. The LPE chain has persistence length of 3nm and spacer of 0.7nm, (a) complex with 2G3 ethylenediamine cored dendrimers (b) 2G3 ammonia cored dendrimers.

Table3.4: Analytical model results for the interaction between 2 G3 dendrimers and LPE chain of length L=80nm.

G3 ethylenediamine cored dendrimer				G3 ammonia cored dendrimer			
L_B/b	l_{opt}(nm)	D'=D2R (nm)	$Z^*_{complex}$	L_B/b	l_{opt}(nm)	D'=D2R (nm)	$Z^*_{complex}$
0.99	14.2	25.8	11.71	0.99	12.5	27.5	6.14
1.00	19.9	20.1	3.57	1.00	22.7	17.3	-8.43
1.01	24.6	15.4	-3.14	1.01	28.4	11.6	-16.57
1.03	28.2	11.8	-8.29	1.03	31.6	8.4	-21.14
1.07	34.2	5.8	-16.89	1.07	35.7	4.3	-27
1.14	37.2	2.8	-21.14	1.14	37.7	2.3	-29.86
1.21	38.1	1.9	-22.43	1.21	38.4	1.6	-30.86
1.29	38.6	1.4	-23.14	1.29	38.7	1.3	-31.29
1.36	38.8	1.2	-23.43	1.36	38.9	1.1	-31.57
1.43	39	1	-23.71	1.43	39	1	-31.71
2.14	39.3	0.7	-24.14	2.14	39.3	0.7	-32.14
2.86	39.2	0.8	-24	2.86	39.2	0.8	-32
3.57	39.2	0.8	-24	3.57	39.1	0.9	-31.86

Table3.5: Analytical model results for the interaction between 2 G6ethylenediamine cored dendrimers and LPE chain (541bps), L=184nm, the dendrimers considered as a penetrable sphere with radius R=3.5nm.

pH	Z	L_{iso}	l_{opt}(nm)	Diff (nm)	$Z^*_{complex}$	Z*/Z	D(N,l) (nm)	D'=D2R (nm)	(D'+Diff)N/L	$l_{opt}/2\pi R$
5.5	405	283.5	76	-207.5	296.43	0.73	23	16	-2.08	3.46
7	256	179.2	87.7	-91.5	130.71	0.51	11.3	4.3	-0.95	3.99
8.5	160	112	82.2	-29.8	42.57	0.27	16.8	9.8	-0.22	3.74

Chapter Four

Conclusions and Future Work

Chapter Four

Conclusions and Future work

Through this study we have estimated the optimal wrapping length of LPE chain around the dendrimer, and applied description to the complex at different systems. This study shows that as the Bjerrum length increases the optimal wrapping length increases. The degree of this effect is different according to the dendrimer generation for example G3ammonia cored less effected than G4ammonia cored. This is due to the variation in charge and radius; also this difference has been obvious in G2, G3 ,G4 ethylenediamine cored . The optimal wrapping length begins to decrease with increasing persistence length, the degree of this variable change according to the type and generation of dendrimer.

The optimal wrapping length begins to increase as the salt increases but at higher salt concentration the optimal length becomes almost fixed. This effect is more clear at small generation of dendrimers and becomes less significant as the generation of dendrimer increased The salt's effect is influenced by Bjerrum length value as the Bjerrum length increased the optimal length increases significantly.

We also have studied the effect of pH on the complex because it has a great influence on the radius, the charge of dendrimer and on the mechanism of LPE release. we have shown that as pH value increases the charge of dendrimers decreases leading to decrease in the optimal wrapping length. Our results are in good agreement with the results of the previous studies of Qamhieh.

Also we have studied the aggregate between LPE chain and two dendrimers, we proved that the number of LPE chain turns around the dendrimer increases as the radius of the dendrimers decreases. Through our study we have concluded that as the optimal wrapping length increased the linker is decreased, also we noted that as the length of the chain increases the optimal length increases and linker decreases. This effect has the same pattern for different generations with small variation in value due to the differences in charge and radius from one generation to another.

All our conclusions were in good agreement with previous computer simulation performed by Lyulin and the theoretical study done by Qamhieh et al, on ion-penetrable sphere model.

All this information we get from the soft sphere model provide huge understanding of the complexation which support using the PAMAM dendrimer – LPE chain complex in gene therapy or in any other applications. The model provide an excellent method to understand the behavior of the complex at different conditions, and can be applied to get more information by entering any new variable such as the study of new type of dendrimers.

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دراسة تحليلية للرابطة المتكونة بين الحمض النووي ومركب الدندريمر

إعداد: أماني باسم محمود صباح

إشراف: د. خولة قمحية.

الملخص

إرتكزت هذه ة الدراسة على دراسة الرابطة المتكونة بين الحمض النووي (LPE chain) و بين الدندريمر على اعتباره كرة قابلة للإختراق من قبل الأيونات المحيطة به (Ion-penetrable sphere) حيث شملت الدراسة أجيالا مختلفة من الدندريمر (G2) و(G3)و(G4) و(G5) و(G6), من خلال استخدام نموذج نظري تم تصميمه من قبل (Qamhieh,et al,2009), و لقد تمت مقارنة النتائج مع نتائج دراسات سابقة على نفس النموذج و لكن على أجيال مختلفة من الدندريمر , حيث أظهرت النتائج توافقاً مع هذه الدراسات و مع نتائج مجموعة من برامج المحاكاة الحديثة .

لقد تمت دراسة درجة الالتفاف (l_{opt}) ال (LPE chain) حول الدندريمر لكل من الأجيال السابقة في أنظمة مختلفة, حيث وجد أن درجة التفاف تزداد بزيادة المتغيرات الآتية: تركيز الأملاح في المحلول, و شحنة الدندريمر, و طول (LPE chain), و تقل بزيادة صلابة ال(LPE chain) .

تم دراسة العلاقة بين ال (LPE chain) و اثنان من الدندريمر وهذا التجمع يعرف باسم (Aggregate), و قد أظهرت الدراسة أن معدل الالتفاف ال (LPE chain)حول الدندريمر يزداد بزيادة معدل النقصان في قطر الدندريمر أثناء الالتفاف حوله مما يزيد من عدد لفات ال (LPE chain)حول الدندريمر. وكذلك تم دراسة تأثير تغير طول ال (LPE chain) على درجة الالتفاف (l_{opt}) و على تكون ما يعرف بال (Linker) و قد تبين انه بزيادة طول ال (LPE chain) فأن درجة الالتفاف تزداد و يقل تكون ال (Linker).