Fatma Ebru KOÇ

M.S. Thesis In Genetics and Bioengineering

January 2012

THE INVESTIGATION OF JEFFAMINE® CORED PAMAM TYPE DENDRIMERS AS POTENTIAL DRUG CARRIERS FOR MODEL DRUGS

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APPROVAL PAGE

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Halil Rıdvan ÖZ

Head of Department

This is to certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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January 2012

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ABSTRACT

In this study, the effect of Jeffamine® (polyoxypropylene triamine) cored Polyamidoamine (PAMAM) type dendrimers were investigated on solubility of model drugs. One of the model drugs were used also for *in vitro* release rate and antibacterial activity. For this purpose, Six model drugs (three NSAIDs (Ketoprofen, Ibuprofen, Diflunisal); (an antibiotic (Sulfomethoxazole)); (Naproxen and L-Histidine) were selected. All model drugs are hyrophobic and have low solubility in water. In our experiments, Jeffamine® cored PAMAM type dendrimer was used in different core size, generations and concentrations.

Results showed that Jeffamine® cored PAMAM dendrimers have potential to significantly increase the solubility of model drugs. In addition, higher solubility of model drugs was observed than that of ethylenediamine cored PAMAM dendrimers that reported in literature with same drugs. Also, release rate decreased compared with pure

drug. These results indicates that Jeffamine® cored PAMAM dendrimers would be suitable for controlled drug release studies. In addition, antibacterial activity was improved eigth fold for with dendrimer.

Key words: Jeffamine® cored PAMAM; solubility; *in vitro* release; model drug

JEFFAMİN® ÇEKİRDEKLİ PAMAM TİPİ DENDRİMERLERİN İLAÇ TAŞIYICI OLARAK KULLANIMININ ÇEŞİTLİ İLAÇLAR İÇİN İNCELENMESİ

Fatma Ebru KOÇ

Yüksek Lisans Tezi-Genetik ve Biyomühendislik

Ocak 2012

Tez Yöneticisi:Yrd. Doç. Dr. M. Fatih ABASIYANIK

ÖZ

Bu çalışmada, jeffamin® çekirdekli PAMAM tipi dendrimerlerin çeşitli ilaçların çözünürlüğü üzerine etkisi, ayrıca bir ilaç için in vitro salım hızı ile antibakteriyel aktivitesi üzerine etkisi incelenmiştir. Bu amaçla, altı model ilaç seçilmiştir. Bu ilaçların üç tanesi NSAI ilaç(ketoprofen, ibuprofen, diflunisal); bir tanesi antibiyotik(Sufamethoksazol); naproksen ve L-histidin'dir. Seçilen tüm ilaçlar hirofobik(su sevmeyen) ve suda çözünürlüğü düşük olan ilaçlardır. Deneylerimizde, jeffamin® çekirdekli PAMAM tip dendrimerlerin değişik çekirdek büyüklüğü, jenerasyon ve konsantrasyondaki çözeltileri kullanılmıştır.

Deney sonuçlarıyla, jeffamin® çekirdekli dendrimerlerin tüm seçilmiş ilaçların çözünürlüklerini arttırdığı ayrıca, aynı ilaçlarla etilendiamin çekirdekli PAMAM dendrimerlerle yapılmış literatürdeki çözürlük çalışmalarından daha yüksek değerlere ulaşıldığı ortaya konmuştur. *In vitro* ilaç salım hızı çalışmasında ise ilacın tek başına olan ilaç salım hızından daha düşük değerler elde edilmiştir. Bu sonuca göre, kontrollü ilaç salımı çalışmalarında bu dendrimerin kullanımının uygun olabileceği söylenebilir.

Aynı ilaç için yapılan antibakteriyel aktivite testinde dendrimerle beraber yapılan çalışmada tek ilaca göre 8 kat daha etkili sonuca ulaşılmıştır.

Anahtar kelimeler: jeffamin® çekirdekli PAMAM; çözünürlük; *in vitro* salım; model ilaç

Dedicated to suns of my life:

my son and my daughter…

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I want to thanks to my son and my daugher for their patient and understanding. And finally but the most important for me, I am thankfull to my life companion who always stand by me.

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LIST OF SYMBOLS AND ABBREVIATIONS

Bis-MPA	:	poly(2,2-bis(hydroxymethyl)propionic acid
G	:	Generation of a dendrimer
JAPD	:	Jeffamine® Cored PAMAM Type Dendrimer
Jeffamine®	:	poly(oxypropylene triamine)
LbL	:	Layer by Layer
NSAIDS	:	Non-Steroidal Anti-Inflamatory Drugs
PAMAM	:	Poly(amidoamine)
PEG	:	Polyethylene glycol
PEI	:	Polyethylene imine
PPI	:	Poly(propyleneimine)
PLL	:	poly(L-lysine)
SMZ	:	Sulfamethoxazole
UV-Vis	:	Ultravisible Spectroscopy

CHAPTER 1

INTRODUCTION

1.1.DRUG DELIVERY

1.1.1.The Basic Concepts

When a patient takes a drug, for example blood pressure decreases as a resulting biological effect. Because of these types of results, the pharmacological properties of drugs have been investigated by scientists.

Drug delivery is a new and dynamic field in bioengineering. The purpose of any delivery system is to carry of a therapeutic compound to its site of action with a determined rate and concentration. The most of drug molecules are hyrophobic and have low-water solubility. Because of this, they cause a lot of side effects and their activities are low. These situations limits the clinical applications of drugs. For these reasons a wide variety of structures and materials are used to increase the solubility of drugs in water.

Ideally, a drug delivery system could deliver the certain amount of drug to the site of action at the certain rate in order to maximize the desired therapeutic response [1].

1.1.2.Types of Delivery Systems

Modern drug delivery studies have been begun with the use of polymer carriers by Folkman and Long who discovered that drug molecules (hyrophobic and small size) diffused through the wall of silicone tubing at a controlled rate in 1964 [2,3].

During the past decades, a large number of drug delivery systems have been designed to take suitable advantages from polymer using[4]. Significant efforts have been devoted also to explore nanotechnology based delivery systems by using multiple disciplines of chemistry, biology, and engineering [5].

Polymeric Drug Delivery Systems can be seperated in 6 topics:

1.Nanospheres and Nanocapsules

2.Liposomes and Polymersomes

3.Linear Polymers

4. Micelles

5.Dendritic Polymers

6. Layer by Layer Systems

1.1.2.1 Nanospheres and nanocapsules

Nanoparticles are the colloidal particulate systems with a size ranging between 1-1000 nm. According to the arrangement of drug and polymer matrix, nanoparticles can be classified into two types: nanospheres and nanocapsules (figure1.1). In nanospheres, drugs are either adsorbed or entrapped inside the polymeric matrix. In nanocapsules, drugs are confined to the inner liquid core while the external surface of nanoparticles is covered by the polymeric membrane [6].

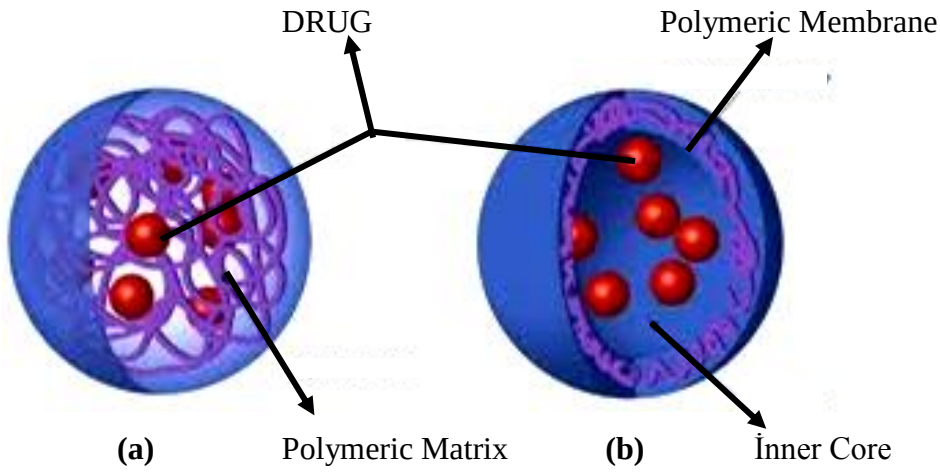


Figure 1.1. (a) Nanosphere; **(b)** Nanocapsules [7].

1.1.2.2.Liposomes and Polymersomes

Liposomes are used for drug delivery due to their unique properties: A liposome encapsulates a aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and

hydrophilic molecules (figure 1.2) To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents [8].

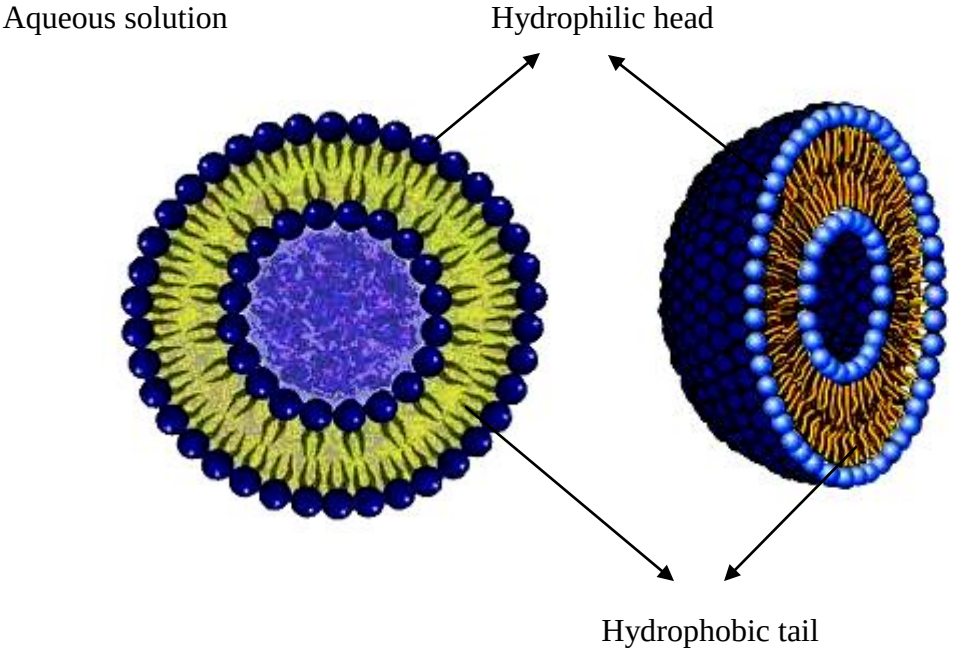


Figure 1.2. Structure of Liposomes [9, 10].

Polymer vesicles having a liposome-like structure with a hydrophobic polymer membrane and hydrophilic inner cavity are called polymersome [8] (figure 1.3). The polymersomes offer some advantages over liposomes, not only in vesicle stability but also in the regulation of membrane thickness. Current polymersome research involves quite diverse fields such as drug delivery system, transfection vectors, protective shells for sensitive enzymes, and microreactors [11,12].

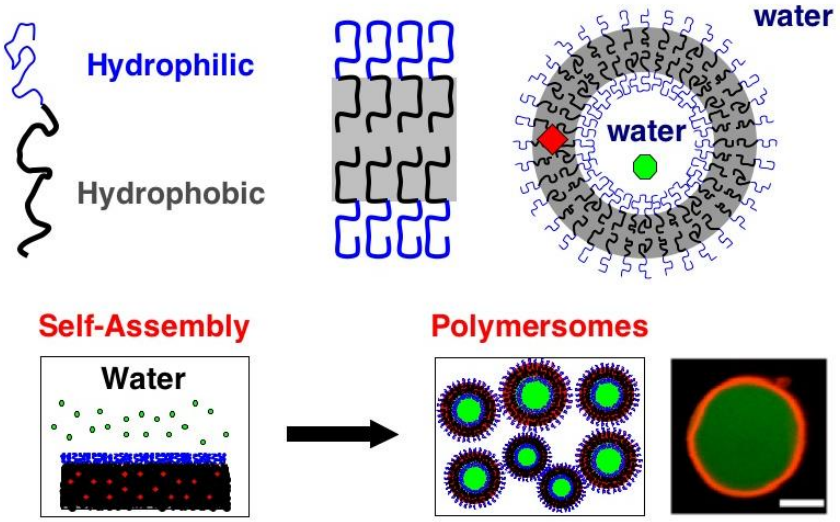


Figure 1.3. Schematic depicting the self-assemblyofpolymersome [13].

1.1.2.3.Linear Polymers

Linear polymers are the simplest polymeric architectural forms (figure 1.4). They have two advantages: First, their structures of 5-15 nm in size in good solvents depending on molecular weight and polymer-solvent interactions; second, the ability of tailored multivalency by introducing function along the polymer backbone. Drugs molecules are bond by conjugating with polymeric backbone.

Linking drugs onto polymers for drug-targeting purpose was first reported in the 1950s. A general model for polymer-drug conjugation was proposed by Ringsdorf in 1975 [14] (figure 1.5).

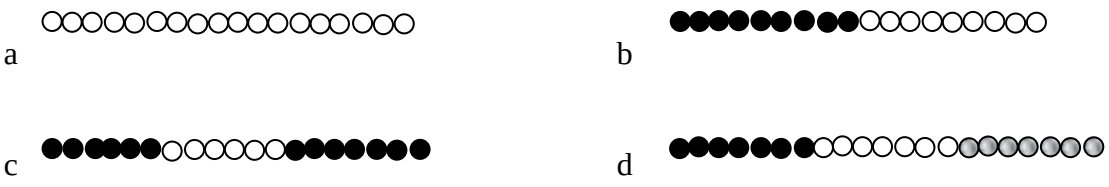


Figure1.4.(a) Homopolymer; (b) AB-type diblock cppolymer; (c)ABA-type triblock copolymer(d) ABC-type triblock copolymer[14].

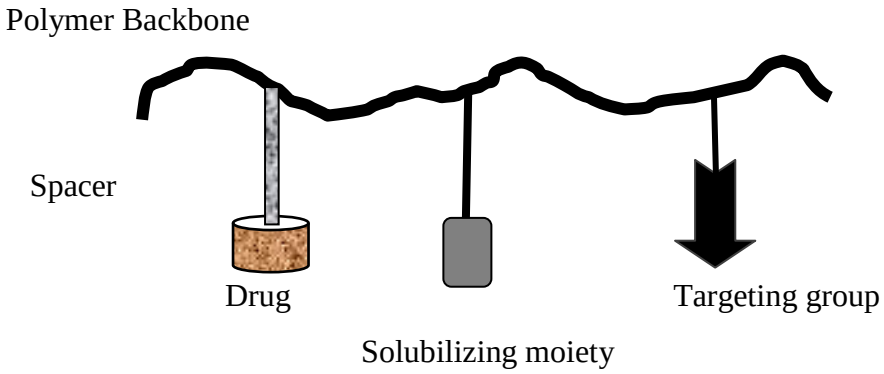
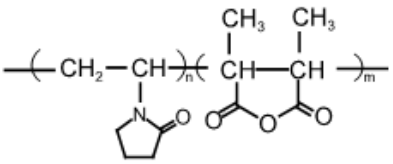


Figure1.5. Ringsdorf model of polymer-drug conjugate; main elements: polymer backbone, drug, spacer, solubilizing moiety, targeting group[14].

Vinyl polymers, polysaccharides, poly(amino acids), proteins, and poly(ethylene glycol) (PEG) are mostly used for carrying drugs[15,16].

Some examples of this kind of polymer are poly(vinylpyrrolidone-co-dimethyl maleic anhydride); poly[(N-hydroxyalkyl) glutamine];Poly(ethylene glycol) (Figure1.6). The functional side groups of these polymers offer the possibility of coupling with drug molecules[17,18].

(a)



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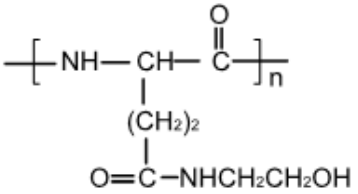

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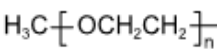


Figure1.6.(a) Poly(vinylpyrrolidone-co-dimethyl maleic anhydride);
(b) poly[(N-hydroxyalkyl) glutamine]; **(c)** Poly(ethylene glycol)

1.1.2.4. Micelles

Linear amphiphilic block copolymers play an essential role in carrying drugs on a nanoscale level [19]. Amphiphilic block polymers have both hydrophilic (water) and hydrophobic (oil) blocks in the same polymer chain. They can build spherical polymeric assemblies in aqueous solution, called "polymeric micelles". Studies on polymeric micelles were begun in 1960s. But the first application of drug carrier was reported in 1984 by Bader et al. [20] and Pratten et al. [21].

There are three major types of micelle delivery systems ; (1) common block copolymer micelle, (2) drug-conjugated block copolymer micelle, and (3) block ionomer complex micelle (Figure1.7).

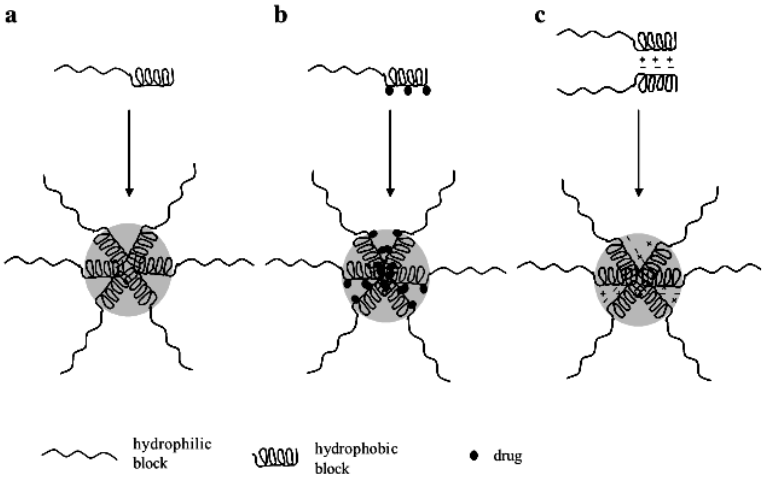
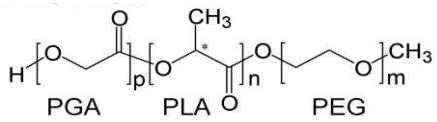


Figure 1.7. Three major types of micelles based on linear block copolymer: **(a)**common block copolymer micelle, **(b)** drug-conjugated block copolymer micelle, and **(c)** block ionomer complex micelle [22] .

PEG is most often used as a hydrophilic segment because of its flexibility, nontoxicity, and hydrophilicity. However, the options available for the hydrophobic block are much broader. For example, the AB-type block polymer PEG-b-polyester, such as PEG-b-poly(lactide-co-glycolide) (PLGA), and PEG-b-poly((-caprolactone) (PCL), is a popular family of block polymers used for drug delivery (figure1.8) [22].

(a)



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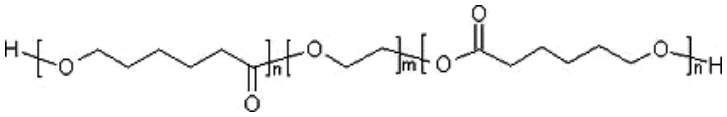


Figure 1.8. (a) PEG-b-poly(lactide-co-glycolide) (PLGA);
(b) PEG-b-poly((-caprolactone) (PCL).

1.1.2.5. Dendrimers

Dendrimers are highly branched, monodisperse and three-dimensional macromolecules with symmetrical, nanometer sized architecture. They are prepared by multistep synthetic procedures[23].The term dendrimer is derived from the Greek words dendri- meaning ‘‘tree-like’’ and meros meaning ‘‘part of’’[24]. Polypropylenimine (PPI) is the first dendrimer was synthesized by Vögtle et al. in 1978 [24] But it was synthesized in only low generation. Newkome et al. [26] and Tomalia [25] synthesized dendrimers at higher generations with well-defined structures in the mid-80s. Properties of these dendrimers such as uniform size, water solubility, modifiable surface functionality and available internal cavities make them attractive for biological and drug-delivery applications [30].

Nowadays, there are over 100 different dendrimer structures [31]. Several of the most commonly dendrimers are shown in figure 1.12 and include Tomalia’s polyamidoamine (PAMAM), [24] Denkewalter’s poly(L-lysine) (PLL),[29] Newkome’s polyamide, [25] Grinstaff’s polyester (PGLSA-OH), [54] Vögtle’s polypropylenimine (PPI), [26] and Hult’s poly(2,2-bis(hydroxymethyl)propionic acid (bis-MPA) [30] structures.

Dendrimers consist a central core, the interior, and the shell. The core affects the 3D shape of the dendrimer (i.e.spheric, ellipsoidic, or cylindric scaffolds) (figure 1.10). The interior affects the host–guest properties of the dendrimer. The surface of the dendrimer can be further polymerized or modified with functional peripheral groups.

Both the core and the number/type of interior branching units affect the overall dendrimer morphology. Because dendrimer diameters increase linearly while the number of surface groups increases exponentially for each generation (figure 1.9). Hence, dendrimers at low generations are usually flexible and open, while dendrimers at higher generations form more dense, three dimensional shapes. A dendrimer's generation number also has an effect on the rigidity of the overall structure [33].

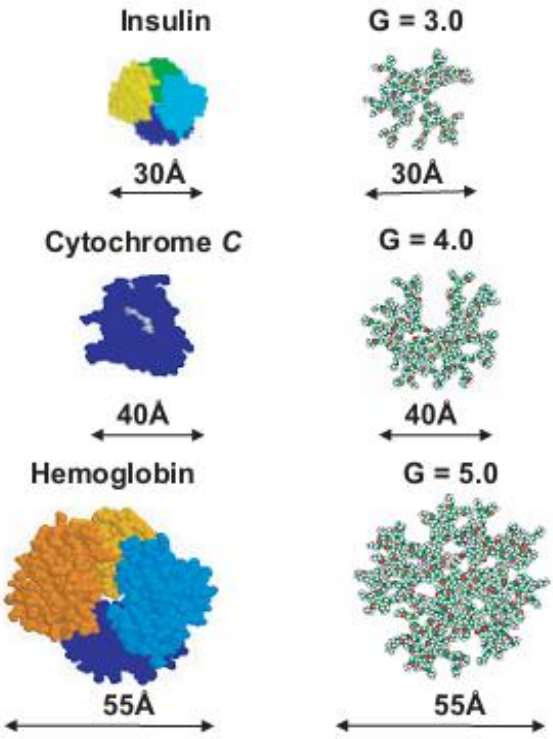


Figure1.9. Poly(amido-amine) (PAMAM) dendrimer generations 3,4,and 5 closely match in size and shape insulin (30 Å), cytochromeC (40 Å), and hemoglobin (55 Å)[32].

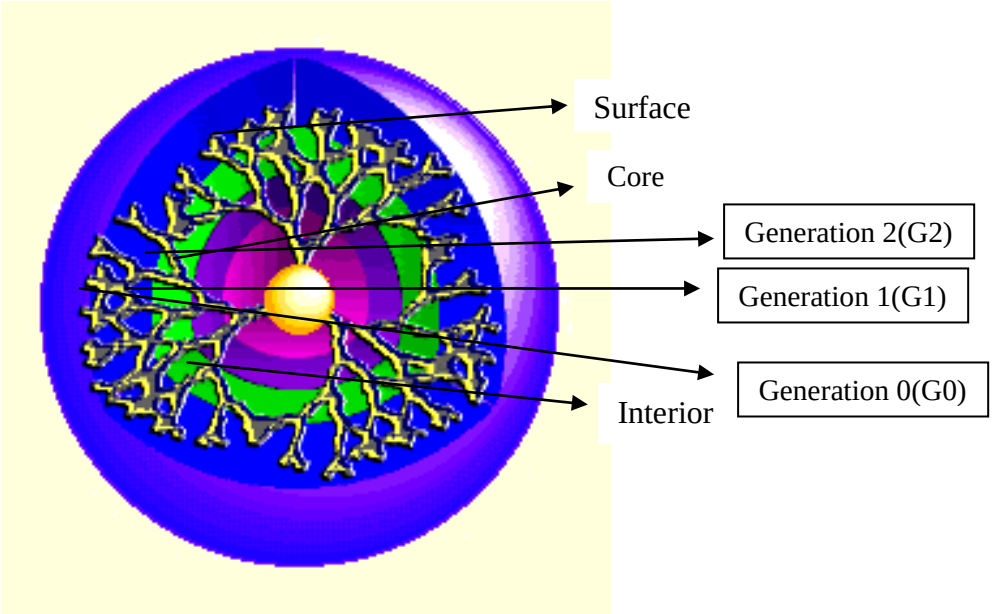


Figure 1.10. Three dimensional projection of dendrimer core-shell architecture [33].

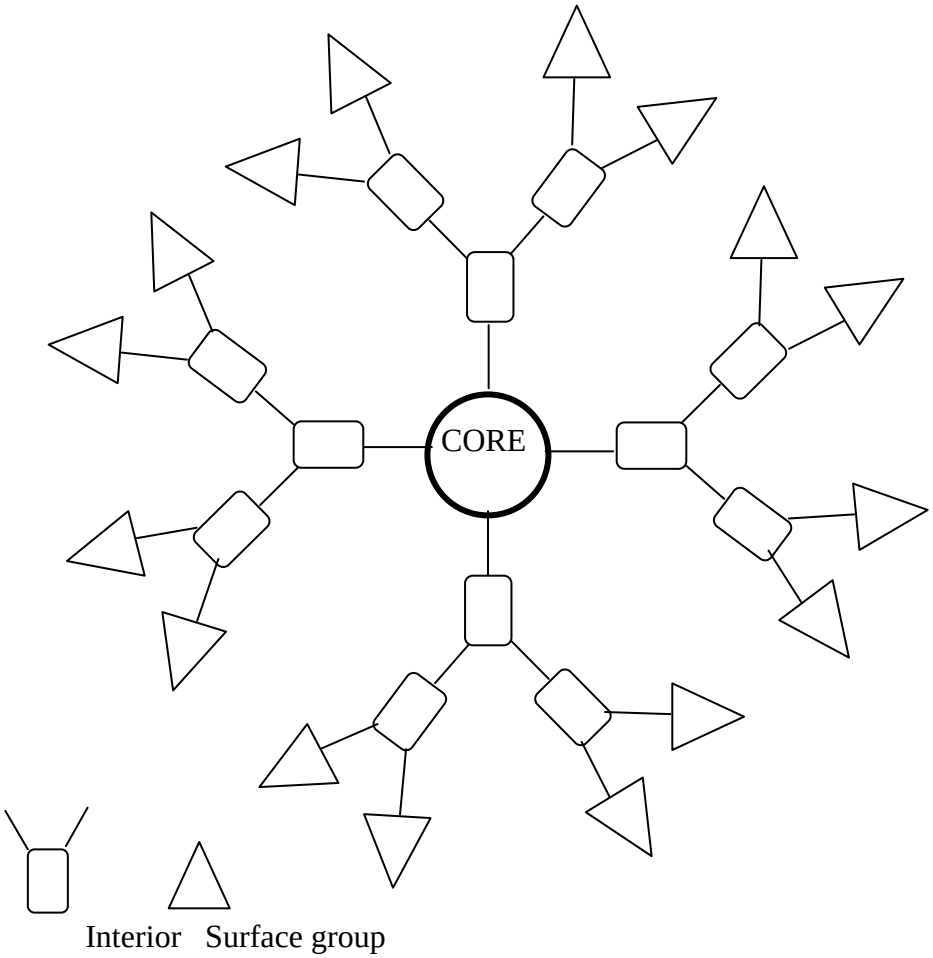
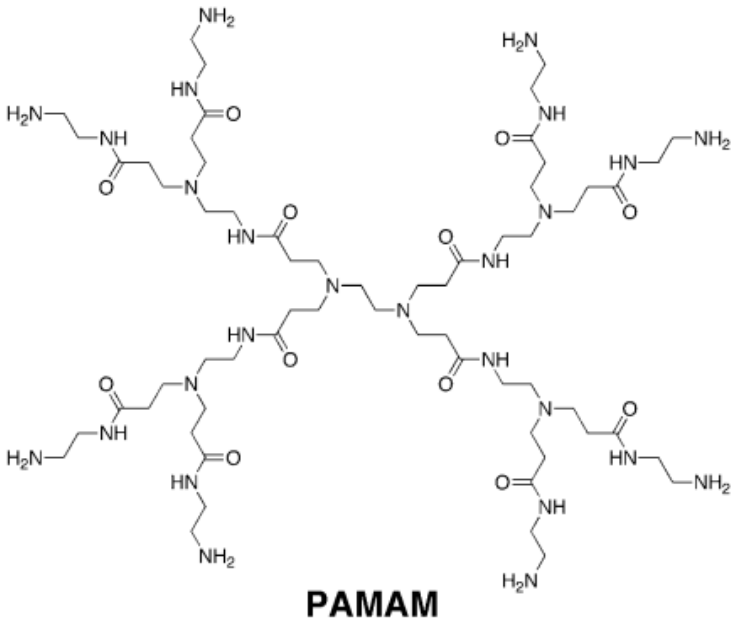
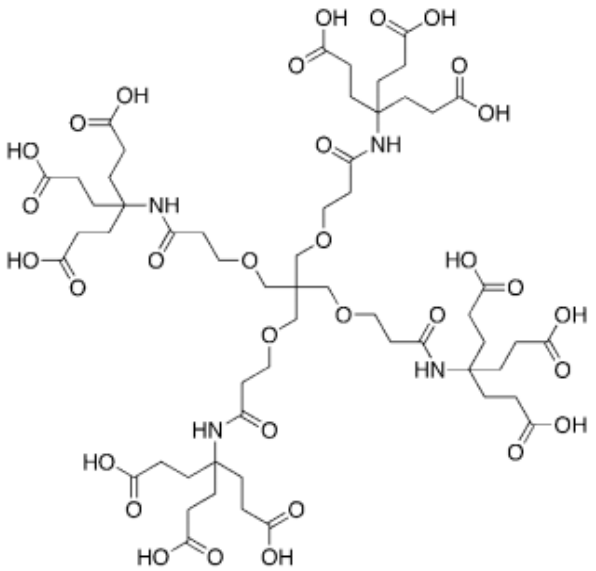


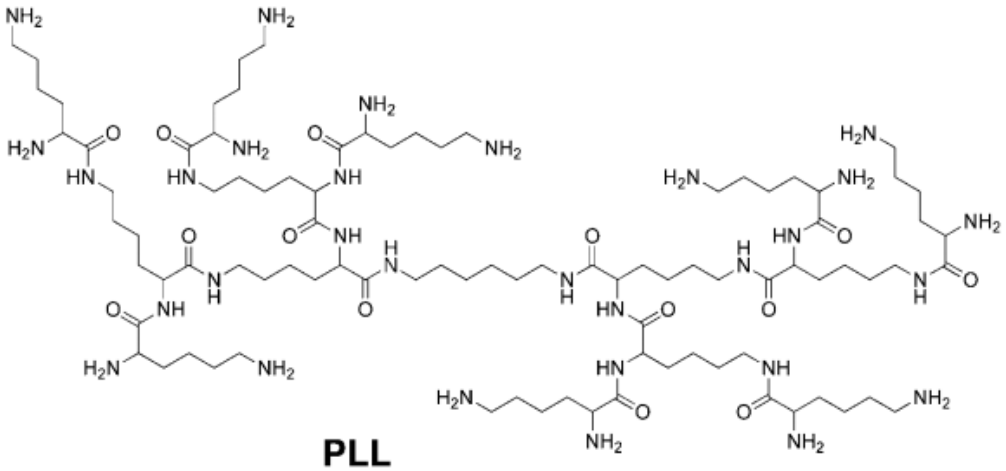
Figure 1.11. Schematic structure of dendrimers.

Dendrimer structures are affected by ionic strength and pH, with changes depending on the type of charged group at the dendrimer surface. In amine-terminated PAMAM, globular, loosely compact structures are observed at high pH while the extended conformation dominates at low pH (≈5) due to electrostatic repulsions of the protonated tertiary amines (pKa ≈5) at the interior of the dendrimer and the primary amines (pKa ≈ 9–11) at the surface [35]. This conformational change affects the endosomal escape of dendrimers following cellular uptake. At physiological pH (7.4) only the primary amines are protonated but after exposure to the endosome environment (pH ≈ 5), the tertiary amines are protonated and the dendrimer conformation change causes endosome rupture. Conversely, for carboxylate terminated PPI dendrimers, small angle neutron scattering studies have shown that at both low (< 4) and high (> 11) pH, the dendrimers displayed an extended conformation due to electrostatic repulsions of either the protonated internal amines at low pH or the deprotonated carboxylic acids on the periphery at high pH. At a pH ≈ 6, however, PPI dendrimer display condensed, backfolded structure due to intramolecular hydrogen bonding of the zwitterionic structure [36].

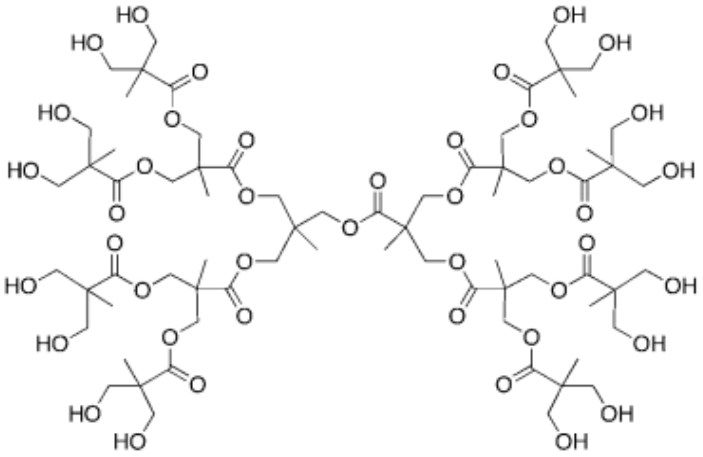




Polyamide



PLL



bis-MPA

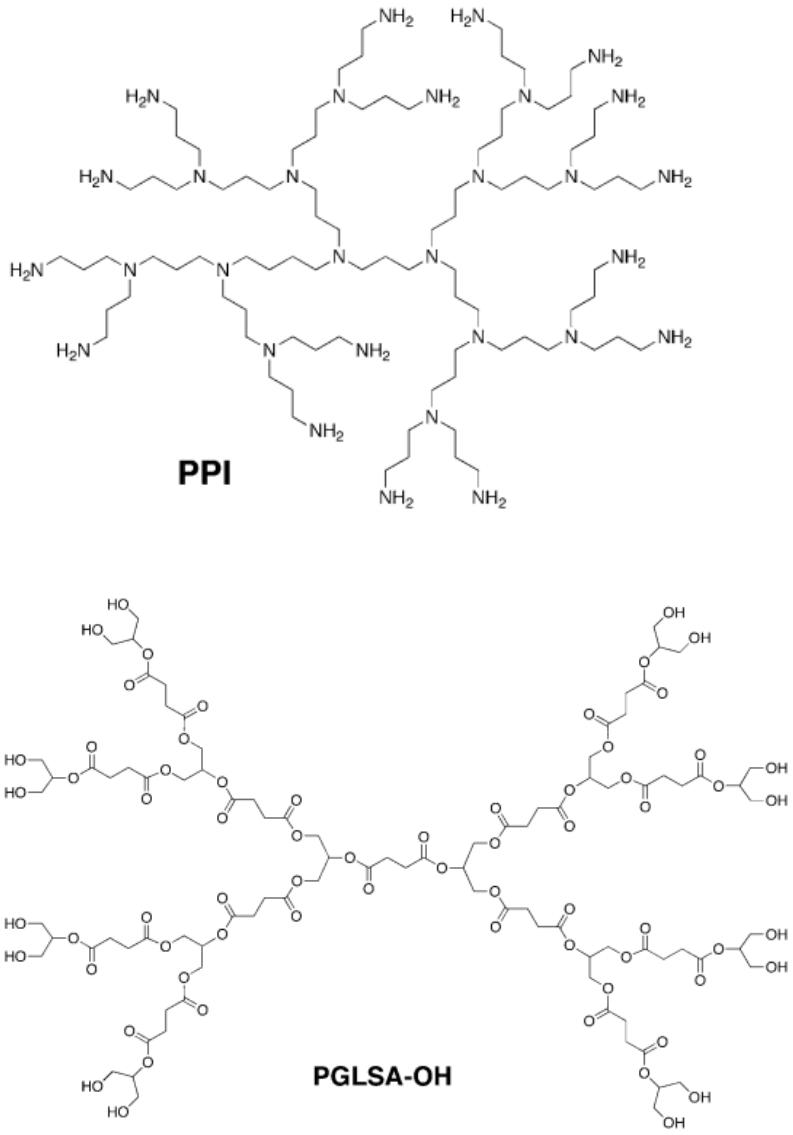


Figure 1.12. Chemical structures of several commonly used, commercially available dendrimer structures

1.1.2.5.1.General Pathways of Dendrimer Synthesis

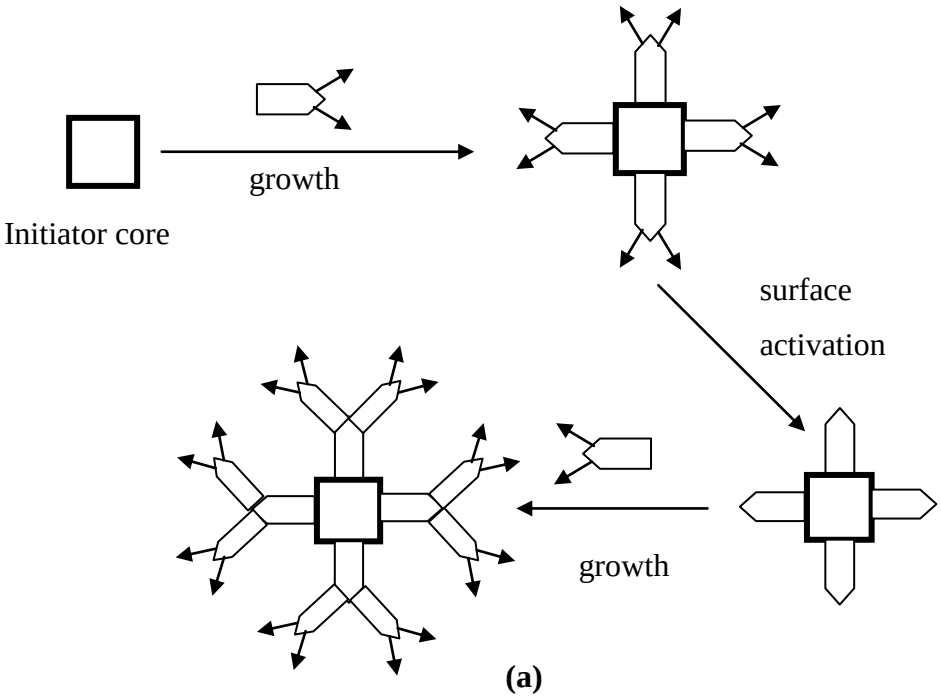
Most dendrimers are synthesized by two main methods; convergent or divergent route, as depicted in Figure 1.12. Each method has its own advantages and disadvantages. The initial synthesis of dendrimers was pioneered by Tomalia et al.,[25] Newkome et al.,[26] and Vogtle et al.[27] and proceeded by divergent routes. In this type of synthesis there are two steps;

1) the activation of the functional surface groups and 2)the addition of branching monomer units. The reaction starts with the initiator polyfunctional core and the first generation monomer units react with the core. Following reactions with the core, the unreactive or protected groups are activated for further reactions with additional

monomer units (37,38). The divergent route can be used for the synthesis of a broad spectrum of dendrimer structures but can be limited by incomplete reaction of the groups leading to the defects in the branching. To overcome this limitation, the monomer unit is often added in excess, thus requiring purification after each step. However, such purification cannot eliminate all incomplete byproducts (38).

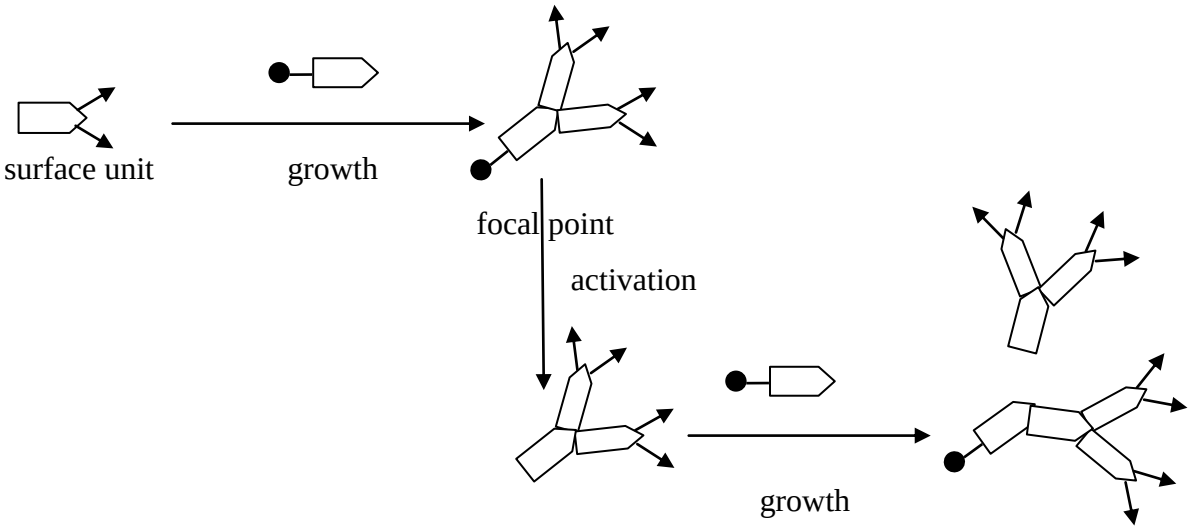
The second commonly used method for dendrimer synthesis is the convergent approach, pioneered by Frechet et al.[39]. In this method; the reaction starts with surface group and then a dendron that reacts with a suitable core to complete the synthesis.

The convergent approach is advantageous because only a limited number of active sites are present per reaction, reducing structural defects in the product. As a result, higher percentages of defect-free product can be obtained per generation and can be isolated from the byproducts. However, the convergent approach is generally used to form only lower generation structures because steric hindrance is encountered when large dendrons are reacted with a small core to form dendrimers with higher generations. Still, because most dendrimers used for biomedical applications are fourth generation analogues, this has not been a limitation [39].



(a)

: reactive branching unit : passive functionality



(b)

Figure 1.13.Dendrimer synthesis routes **(a)** divergent; **(b)** convergent.

1.1.2.5.2.Dendrimers as drug carriers

Drugs can be carried to the target by dendrimers with different ways; encapsulation and conjugation.

1.1.2.5.2.1.Encapsulation

Low water solubility of most drugs is the major problem. Solubility of dendrimers depends on their structure. Dendrimers have been designed with water-soluble external groups and hydrophobic interiors in such a way that they are able to encapsulate hydrophobic drugs [42-45]. Alternatively, positively or negatively charged dendrimer can electrostatically bind drugs bearing opposite charges [46-49]. Drug molecules can be loaded by dendrimers with different ways such as ionic bonding, hydrogen bonding, van der Waals interactions, π bonding, hydrophobic interactions. And also, Small drug molecules are encapsulated mostly by dendrimer interior (figure 1.14).

A classic example is the anti-inflammatory drug ibuprofen for which 78 molecules were found to complex *G*4-PAMAM dendrimer at the amine dendrimer groups through electrostatic interactions with the carboxy groups of the drug. In vitro release was shown to be slow compared to the free drug. The drug-dendrimer complex

was found to enter A549 cells much more rapidly than free ibuprofen, suggesting efficient drug carrier into the cell [50,51].

The water-insoluble anticancer drugs camptothecins were encapsulated in *G*4.5 carboxylate-terminated polyester dendrimer [52-54] Poly(glycerol succinic acid) dendrimers (PGLSA dendrimers) were also investigated for their capacity to encapsulate camptothecins. *G*4-PGLSA-CO2Na (unlike *G*4-PGLSA-OH) was successfully used in the case of 10-hydroxycamptothecin, and their exposure to MCF-7 human breast cancer cells led to significant increase in toxicity with less than 5% of viable cells at a concentration of 20 μM [55].

The advantage of noncovalent drug-dendrimer interactions, however, is the higher solubility of water-insoluble drugs than with conjugates. Furthermore, conjugation allows a higher drug payloads [56,57].

1.2.2.5.2.2.Conjugation

Dendrimer-drug conjugates are another application for drug delivery. Sometimes, encapsulated drugs which can be released before reaching the targeted cell in drug-dendrimer complexes. Drug-dendrimer conjugates are superior for these situations, because of the stronger interaction between drug and its carrier drug can be specifically target to the cell and then the multiple drug molecules are released from a single dendrimer-drug conjugate by pH change at the cell environment. They decrease nonspecific toxicity, optimize biodistribution, and increase circulation time in blood. Its half life in plasma is increased as well as drug resistance [58]. Dendrimer-drug conjugates rapidly penetrate in to the cells and cytoplasm [59,60].

Methotrexate delivery to CCRF-CEM human acute lymphoblastoid leukemia and CHO Chinese hamster ovary cell lines was achieved by PAMAM methotrexate conjugates. It was more efficient compared to the free drug. The decrease of lyposomal residence time of the cationic PAMAM subsequent to drug cleavage was taken to be responsible for reduced drug release [61].

Noncovalent and covalent drug-dendrimer applications usually increase drug efficiency compared to the free drug, and several drug-dendrimer complexes or conjugates are in early clinical trials [62].

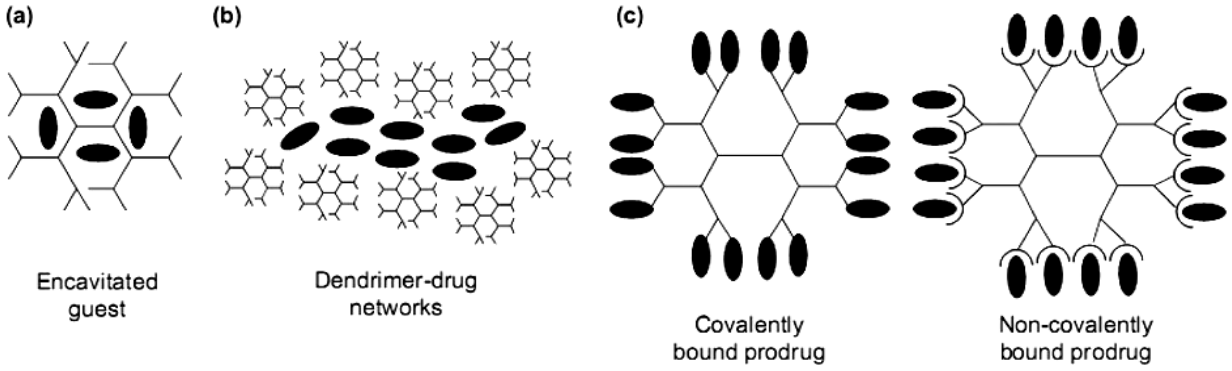


Figure 1.14. Schematic representations of dendrimer drug delivery systems. The darkened oval represents an active substance [63].

1.1.2.5.3. *In vitro* **toxicity**

The toxicity of dendrimers toxicity depends on the nature of dendrimers. For example, cationic dendrimers, such as PAMAM and polypropyleneimine (PPI) (include primary amines groups) dendrimers generally display concentration-dependent toxicity and hemolysis [61-63], but dendrimers containing only neutral or anionic components have been shown to be much less toxic and less hemolytic [68-70]. The cytotoxicity of the cationic dendrimers can be explained by the interactions between negatively charged cell membranes and the positively charged dendrimer surface, enabling these dendrimers to adhere to and damage the cell membrane, causing cell lysis.[64]. The toxicity of cationic PAMAM dendrimers increases with each generation [64,66].

1.1.2.5.4. Degradation

The most widely studied dendrimers, PAMAMs, have amide backbones, they are hydrolytically degradable only under harsh conditions [67], and hydrolysis proceeds slowly at physiological temperatures.

More promising dendrimers are based on polyester backbones in terms of hydrolytic degradability [68-72]. In one example, polyester dendrimers have been carefully designed so that the ester hydrolysis products are nontoxic, natural metabolites [68], whereas in another instance high molecular weight polyester dendrimers and dendronized polymers have been shown to degrade to putative excretable and nontoxic lower molecular weight species [71].

The most biologically relevant triggering mechanisms have employed reactions induced by ultraviolet irradiation or catalytic antibodies [72,73]. Importantly, this disassembly strategy not only results in complete and rapid dendrimer degradation, but also provides a means for release of multiple biologically active species or spectroscopic labels from dendrimer end groups from a single, chemoselective cleavage event. Although the aromatic decomposition products of some of the dendrimers are nontoxic [74], it will be interesting to learn if less hydrophobic aliphatic molecules can be used to increase dendrimer solubility and ensure their biocompatibility.

1.1.2.5.5. Advantages of dendrimers

Liposomes are the most commercial carriers. Because their drug loading capasities are high (10–15,000 drugs/liposome), they can be used in a variety of sizes (50–10,000 nm), they are biodegradable and can be easily modified on their surfaces. However, liposomes are multicomponent, noncovalently associated compared with macromolecules like dendrimers with covalently associated drugs [75].

Polymers are smaller sized carriers (<50 nm) and have a lower payload per particle than liposomes. Polymers manufactured via chemical syntheses are perhaps more easily produced on a large scale, but none are currently approved for use in parenteral drug products [76] because of their nonbiodegradability and high polydispersity.

Properties of dendritic polymers are different significantly from linear polymers. They have a number of beneficial attributes for biomedical applications. Including the following: Dendrimers have high structural and chemical homogeneity; biodistribution and pharmacokinetic properties can be controlled by dendrimer size and conformation; easily modified and ability to be functionalized on their surfaces, drug-loading capasity increase by this way and controlled degradation. However, dendrimers can be prepared

multistep syntheses and these syntheses have high costs[76,77]. In summary, the comparison of properties between dendrimers and linear polymers are given in Table 1.1.

Table 1.1 Properties dendrimers and linear polymers [78].

Property	**Dendrimers**	**Linear Polymers**
Structure	Compact, Globular	Not compact
Synthesis	Careful & stepwise growth	Single step polycondensation
Structural control	Very high	Low
Architecture	Regular	Irregular
Shape	Spherical	Random coil
Crystallanity	Non-crystalline, amorphous materials -lower glass temperatures	Semi crystalline/crystalline materials -Higher glass temperatures
Aqueous solubility	High	Low
Nonpolar solubility	High	Low
Viscosity	Non linear relationship with molecular weight	Linear relation with molecular weight
Reactivity	High	Low
Compressibility	Low	High
Polydispersity	Monodisperse	Polydisperse

1.1.2.6.Layer by Layer (LbL)

The layer-by-layer (LbL) assembly of polyelectrolyte polymer layers on solid surface is a versatile technique that has shown great promise in drug delivery [79-84]. The popularity of this technique in bioengineering from the fact that therapeutics can be incorporated into these multilayer assemblies either directly [85] or within a carrier [86, 87]. In addition, the films are formed nanolayer level, it is possible to achieve nanometer scale precision over the composition and the internal structure of the resultant multicomponent film [88,89]. Together, these factors have facilitated the creation of specialized thin film structures with sophisticated levels of spatial, temporal or active control over the release of therapeutics from the surfaces of macroscopic objects[90].

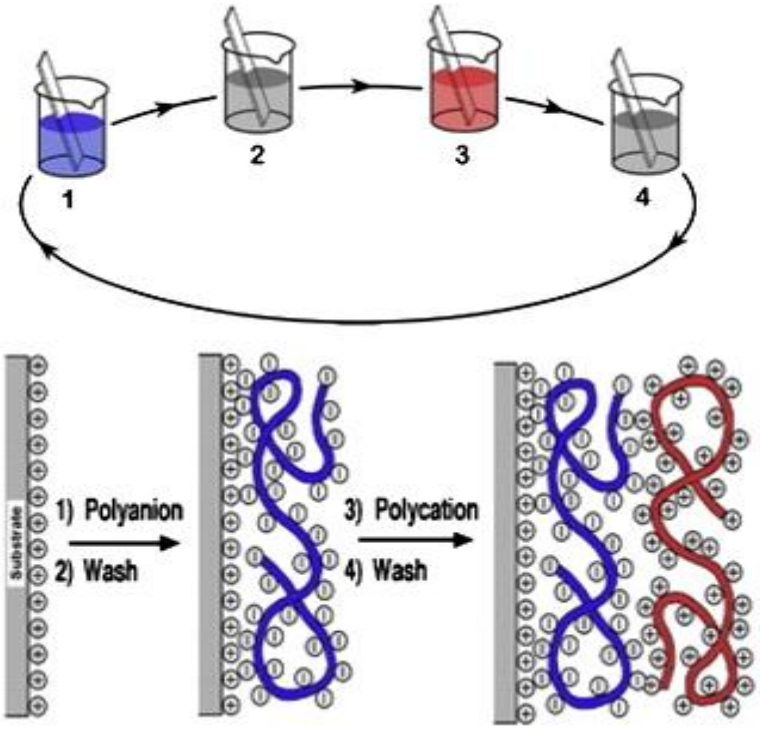


Figure 1.15. Layer by Layer procedure [91].

In Layer by Layer (LbL) approach consists of absorption of polyanions, such as PSS (poly(styrenesulfonate)) and DXS(dextran sulfate), and polycations, like PAH (poly(allylamine hydrochloride)) and PRM (protamine dextran) ,(figure 1.15) [91]. The technique takes advantage of attractive electrostatic forces between charged polymers and oppositely charged surfaces, and film growth is achieved stepwise by the repetitive exposure of substrates to dilute polycation and polyanion solutions. Hydrophilic and positively charged substrates are immersed into the solution of polyanion (negatively charged polymer, for example, PSS) for several minutes. As a result, a thin layer (thickness 1–2 nm) of the polymer is adsorbed on the surface. Charge overcompensation leads to a negative surface recharging. Then, the substrate is washed (a washing step is needed to remove not adsorbed material) and placed into the solution with polycation (positively charged polymer, for example, PAH). The polymer is attached electrostatically to the charged surface. The process can be repeated several times to reach a defined multilayer thickness controlled by layer coating cycling. Layer-by-Layer technique of assembly permits the deposition of thin films on a wide variety of macroscopic, microscopic, and nanoscopic objects [92-98].

1.2.DRUG RELEASE

Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which chemists and chemical engineers are contributing to human health care. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use synthetic polymers as carriers for the drugs. By so doing, treatments that would not otherwise be possible are now in conventional use. Although the introduction of the first clinical controlled release systems occurred less than 25 years ago, 1997 sales of advanced drug delivery systems in the United States alone were approximately $14 billion dollars [99]. All controlled release systems aim to improve the effectiveness of drug therapy [99,100]. This improvement can take the form of increasing therapeutic activity compared to the intensity of side effects, reducing the number of drug administrations required during treatment, or eliminating the need for specialized drug administration (e.g., repeated injections). Two types of control over drug release can be achieved, temporal and distribution control [100].

1.2.1.Methods of Controlled Release

In temporal control, drug delivery systems aim to deliver the drug over an extended duration or at a specific time during treatment. Controlled release over an extended duration is highly beneficial for drugs that are rapidly metabolized and eliminated from the body after administration. An example of this benefit is shown schematically in Figure1.16 in which the concentration of drug at the site of activity within the body is compared after immediate release from 4 injections administered at 6 hourly intervals and after extended release from a controlled release system. Drug concentrations may fluctuate widely during the 24 h period when the drug is administered via bolus injection, and for only a portion of the treatment period is the drug concentration in the therapeutic window (i.e., the drug concentration that produces beneficial effects without harmful side effects). With the controlled release system, the rate of drug release matches the rate of drug elimination and, therefore, the drug concentration is within the therapeutic window for the vast majority of the 24 h period.

Clinically, temporal control can produce a significant improvement in drug therapy. For example, when an opioid pain killer is administered to a patient with terminal cancer, any time that the drug concentration is below therapeutic concentrations the patient experiences pain. A temporally controlled release system would ensure that the maximum possible benefit is derived from the drug. In distribution control, drug delivery systems aim to target the release of the drug to the precise site of activity within the body. The benefit of this type of control is shown schematically in figure 1.17 in which drug concentrations at the site of activity and sideeffect production are compared. There are two principle situations in which distribution control can be beneficial. The first is when the natural distribution causes drug molecules to encounter tissues and cause major side effects that prohibit further treatment. This situation is often the cause of chemotherapy failure when bone marrow cell death prevents the patient from undergoing a complete drug treatment. The second situation is when the natural distribution of the drug does not allow drug molecules to reach their molecular site of action. For example, a drug molecule that acts on a receptor in the brain will not be active if it is distributed by the patient's blood system but cannot cross the blood-brain barrier [101].

A large number of classes of drugs can benefit from temporal or distribution controlled release. These classes include chemotherapeutic drugs [102,104]; antiinflammatory agents [105-109]; antibiotics [108]; hormones [109]; anesthetics [110] and vaccines [111]. Recently, the need to develop new controlled release strategies has been intensified by advances in the design of peptide drugs and emergence of gene therapy. These biotechnology derived agents may dominate the next generation of drug design. However, their clinical success may be dependent on the design of controlled release devices that ensure that the drugs reach their target cells precisely at the required time [100].

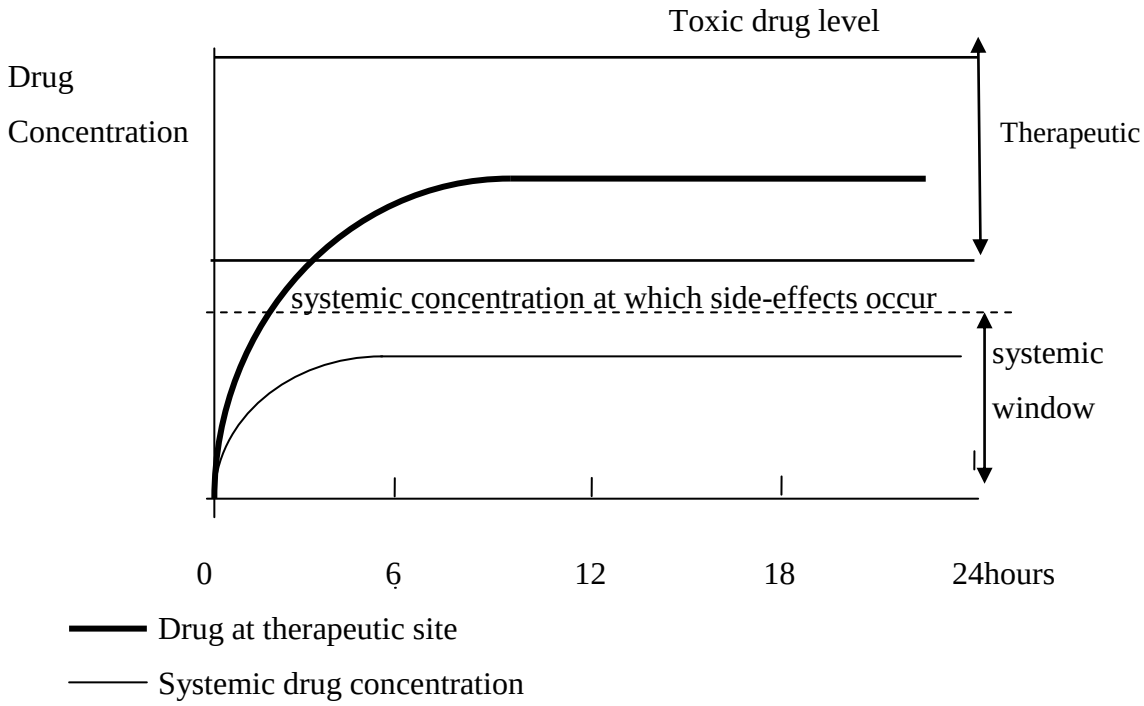


Figure 1.16. The temporal control system[100].

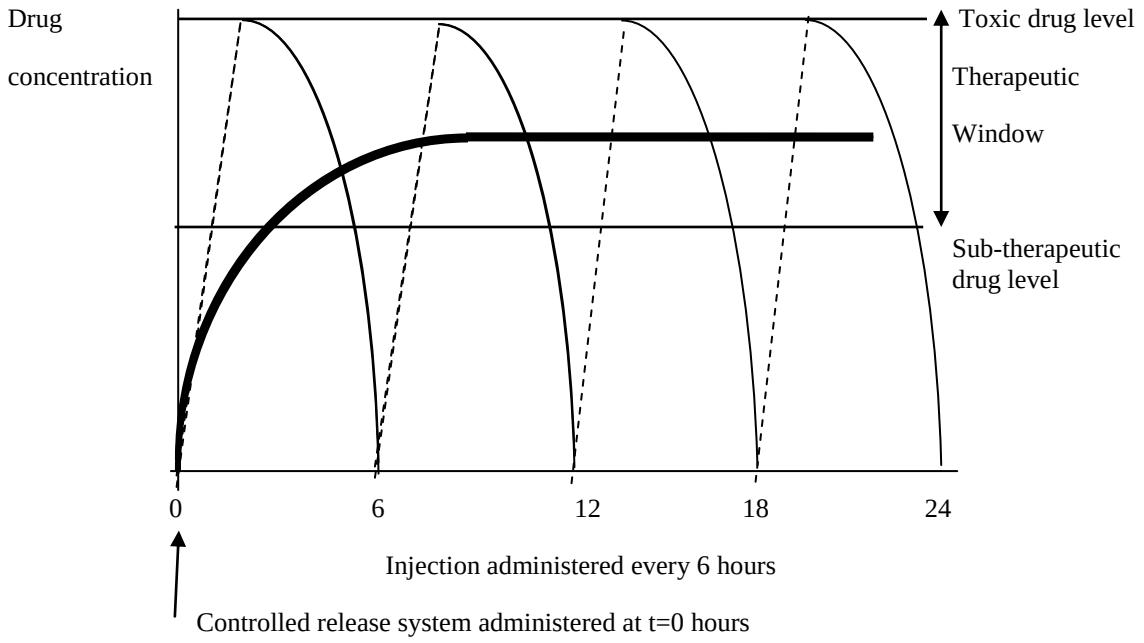


Figure 1.17. The distribution control system[100].

1.3.MODEL DRUGS

1.3.1.Non-Steroidal Anti-Inflamatory Drugs (NSAIDs)

Non-Steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs in the world, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal conditions [112]. Also, they reduce the mortality from colon cancer by about half and constitute the prototypical colon cancer chemopreventive agents [113].But they are used limitily in clinical applications. Because they may cause side effects, for examples; gastrointestinal side effects, renal side effects [113]. The side effects of NSAIDs and their poor solubility have prompted intensive efforts to identify safer alternatives. For example, in order to improve the aqueous solubility of NSAIDs, additional of surface active agents, formation of water soluble salts, increasing the wettability and micronization of drug particles have always been carried out to enhance dissolution, absorption rate and bioavailability of NSAIDs [114-116]. But the proposed methods have not always been sufficient to achieve these goals. Recently, novel macromolecular drug delivery systems have been developed to enhance the solubility of NSAIDs and reduce their clinical toxicity, which promise to be safer alternatives than pure NSAIDs in clinical practice. The attractive features of dendrimers, when compared with traditional linear polymers, make them interesting candidates for the development of novel delivery systems for NSAIDs [117].

The most of the NSAIDs are hydrophobic molecules and they include carboxyl groups. Hydrophobic cavities and hyrophilic exterior surface groups in PAMAM dendrimers make them capable of encapsulating hydrophobic drug molecules and ensure their applications as solubility enhancers of these hydrophobic agents[118].

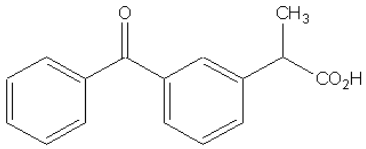
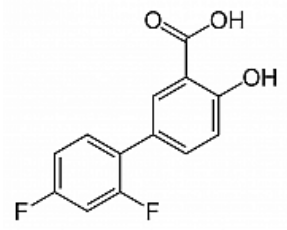
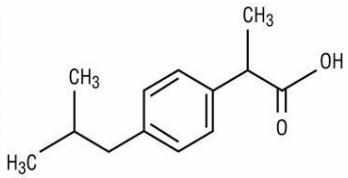
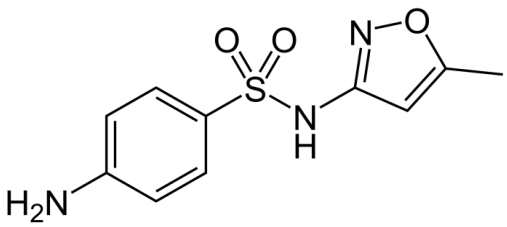
Ketoprofen	**Diflunisal**	**Ibuprofen**
		
$C_6H_{14}O_3$, 254 g/mol	$C_{13}H_8F_3O_3$, 250 g/mol	$C_{13}H_{18}O_2$, 206g/mol
CW= 260 nm, pKa= 4.5	CW= 250 nm, pKa=3.3	CW=221, pKa=5.3

Figure 1.18. Properties of NSAI Model Drugs

1.3.2. Sulfamethoxazole (SMZ)

Sulfonamides are one of the oldest types of antibiotics, and were in use already in the 1930s. They are still among the most commonly used antibiotics both for humans and animals, with 78 tonnes being used in Europeannually (Thiele-Bruhn, 2003). The sulfonamides are the large family of bacteriostatic drugs that are produced by chemical synthesis [119]. However, the clinical use of sulfonamides is limited due to their low solubility in water. On the other hands, their clinical usage is limited because of their extreme low water solubility, rapid elimination in blood, unsufficient association with plasma proteins and several side effect s, which are characterized by fever, skin rash, hepatotoxicity, lymphadenopathy and hematological disorders [120].The poor solubility of sulfonamides restricts their use in topical and parenteral applications. As poor solubility is generally related to low bioavailability, this presents a major challenge during drug formulation. In order to improve the solubility of sulfonamides in water, cyclodextrin-sulfoamides complexes were prepared to enhance dissolution and absorption rate [121,122]. However, high costs and nephrotoxicity on parenteral administration limit the use of cyclodextrins. Moreover, the aqueous solubility of the commonly used cyclodextrin is insufficient to stabilize drugs at therapeutic doses [123].

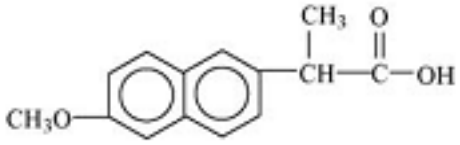


$C_{10}H_{11}N_3O_3S$; 253.28g/mol; CW=265

Figure 1.19. Sulfamethoxazole (SMZ)

1.3.3. Naproxen and L-Histidine

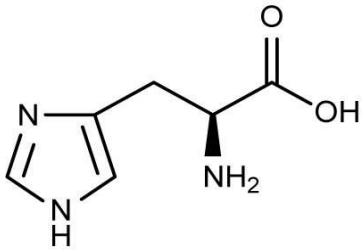
Naproxen is the acidic non-steroidal anti-inflammatory drug (NSAID). It includes carboxyl(-COOH) group (pKa= 4.2), its solubility depends on pH in water [48].



$C_{14}H_{14}O_3$; 230.26g/mol; CW=273nm; pKa= 4.2

Figure 1.20. Naproxen

L-Histidine is one of the 20 standart amino acids in human body. It presents in proteins. Histidine has the imidazole side-chain. Because of this, its pKa approximately 6 (all aminoacids have pKa 7.6) and it is a positively charged weakly basic aminoacid under physiological environment [124].



$C_6H_9N_3O_2$; 155.15g/mol; CW=230nm; pKa=6

Figure 1.21. L-Histidine

CHAPTER 2

MATERIALS AND METHODS

2.1.MATERIALS

Jeffamine® T series products (MW= 440, 3000 and 5000 g/mol) were gifts from HUNTSMAN International, LLC (Turkey). Ketoprofen, Ibuprofen, Diflunisal, Sulfamethoxazole, Naproxen and L-Histidine were obtained from Aldrich. All other chemicals were of analytical grade and used without further purification. Dendrimers were synthesized by Mehmet Şenel from the Department of Chemistry in Fatih University.

The FTIR-ATR spectra (4000–400 cm^{-1}) were recorded with a Bruker spectrometer. NMR spectra were recorded in $CDCl_3$ using a Bruker 400 MHz spectrometer.

2.2.METHODS

2.2.1. Solubility tests of NSAIDs

Firstly, the solution of dendrimers with different generations were prepared at concentrations ($0.25x10^{-3}$M; $0.5x10^{-3}$M; $0.75x10^{-3}$M; $1x10^{-3}$M; $2x10^{-3}$M), respectively. The adequated amount of dendrimers were dissolved in PBS (pH=7.4) solution and their pH values are adjusted. Excess NSAIDs drugs were added to 1 ml of each test solution to ensure drug solution reach the saturation. The solution was mechanically shaken for 24 h at 37°C and then centrifuged at 5000 rpm for a minute. The drug-dendrimer and solely dendrimer solutions were diluted with same ratio by PBS (pH=7.4). The absorbance of NSAIDs test solutions and solely dendrimers solutions at their characteristic wavelengths (260 nm for Ketoprofen, 221 nm for Ibuprofen and 250 nm for Diflunisal) were tested using the SHIMADZU UV-Visible 1700 spectrophotometer. Solely dendrimer absorbances were subtracted from drug-dendrimers test solution

absorbances. These absorbance values were correlated with the calibration curves and the concentrations of drugs were calculated.

2.2.2. Solubility tests of SMZ

Dendrimer solutions were prepared in water with concentrations ($2x10^{-3}$M; $4x10^{-3}$M; $6x10^{-3}$M; $8x10^{-3}$M; $10x10^{-3}$M), respectively. Excess SMZ drug were added to 1 ml of each test solution to ensure the drug solution reaching saturation. The solution was mechanically shaken for 24 h at 37 °C and then centrifuged at 10 000 rpm for 3 minutes. The saturated solutions and solely dendrimer solutions were diluted to proper concentration (500x).The absorbances of SMZ test solutions at their characteristic wavelengths (265 nm) were tested using the SHIMADZU UV-Visible 1700 spectrophotometer. Solely dendrimer absorbances were subtracted from drug-dendrimer test solution absorbances. These absorbance values were correlated with the calibration curves and concentrations of drugs were calculated.

2.2.3. *In vitro* release experiments for SMZ

In vitro release studies of SMZ in presence of 0.01M G (0,1,3,5)-3000 PAMAM dendrimers were investigated. The SMZ was dissolved in dendrimer solution (4mg/mL). Pure SMZ was dissolved in methanol (4mg/mL) as control solution. Each solutions (10mL) were transferred to dialysis bags (M.W. cut off = 1000). The dialysis bags were placed in 1 L beaker which contain 400mL distilled water. Outer phases were stirred continuously (figure 2.1). 1mL of sample was withdrawn from the outer phase and then 1mL distilled water was added to outer phase again, it was repeated during 10 hours at each 30 minutes. The absorbance of samples were measured at 265 nm using UV-Vis spectrophotometer. Concentrations were calculated from SMZ standartgraphic.

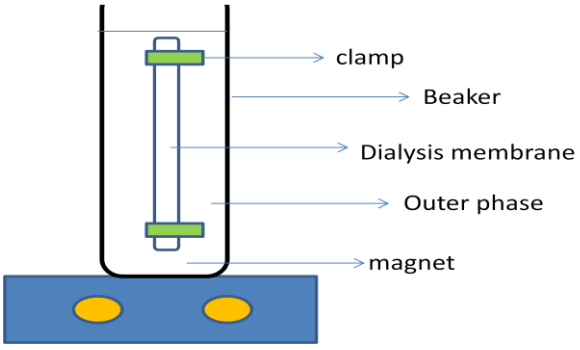


Figure 2.1. Schematic representation of *in vitro* release experimental system

2.2.4. Anti-bacterial activity test for SMZ

These tests were done by Nurdan Bülbül from the Department of Genetics and Bioengineering in Fatih University.

2.3.5. Solubility Tests of Naproxen and L-Histidine

Dendrimer solutions were prepared in PBS with different pH (pH=6.0; 7.0; 8.0) and concentration values ($0.25x10^{-3}$M; $0.5x10^{-3}$M; $0.75x10^{-3}$M; $1x10^{-3}$M; $1.5x10^{-3}$M; $2x10^{-3}$M), respectively. Excess drugs were added to 1 ml of each test solution to ensure drug solution reach the saturation. The solution was mechanically shaken for 24 h at 37 °C and then centrifuged at 10000 rpm for 3 minutes. The drug-dendrimer and solely dendrimer solutions were diluted with same ratio of PBS (pH=6.0; 7.0; 8.0). The absorbances of Naproxen and L-Histidine test solutions and solely dendrimer solutions at their characteristic wavelengths (230 nm for L-Histidine and 273 nm for Naproxen) were tested using the SHIMADZU UV-Visible 1700 spectrophotometer (figure 2.2). Solely dendrimer absorbances were subtracted from drug-dendrimers test solution absorbances. These absorbance values were correlated with the calibration curves and concentrations of drugs were calculated.

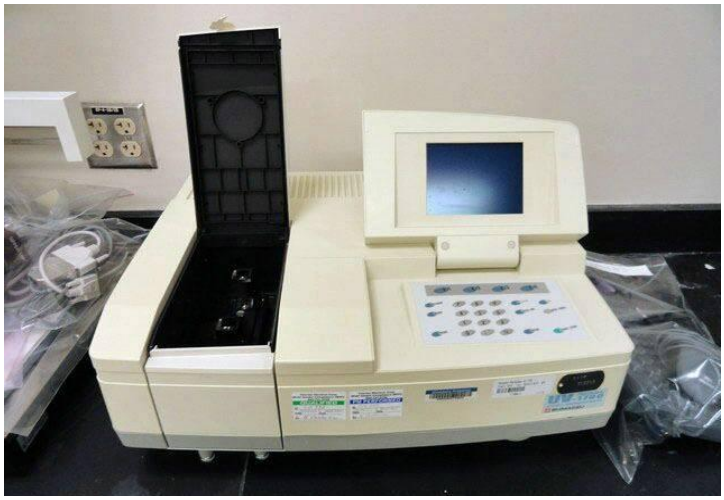


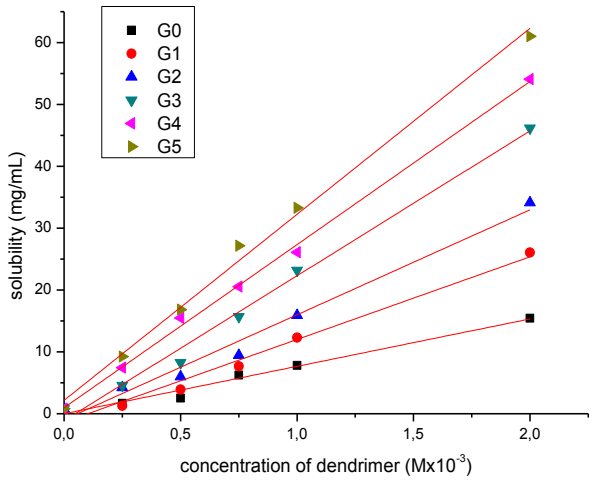
Figure 2.2. SHIMADZU UV-Visible 1700 spectrophotometer.

CHAPTER 3

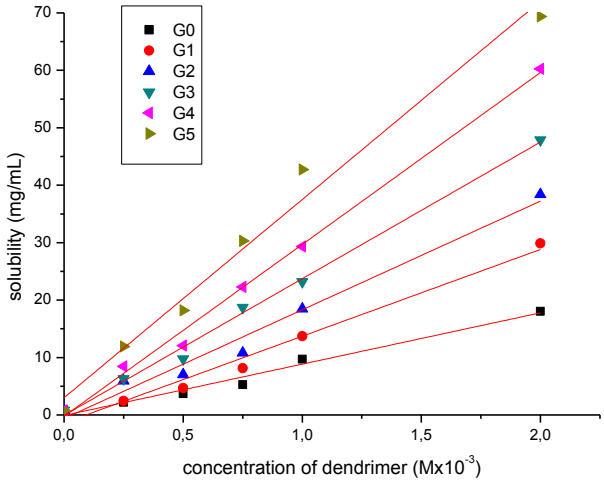
RESULTS AND DISCUSSION

3.1. The Effect of Dendrimers and Core Size on Non-Steroidal Anti-Inflamatory Drugs

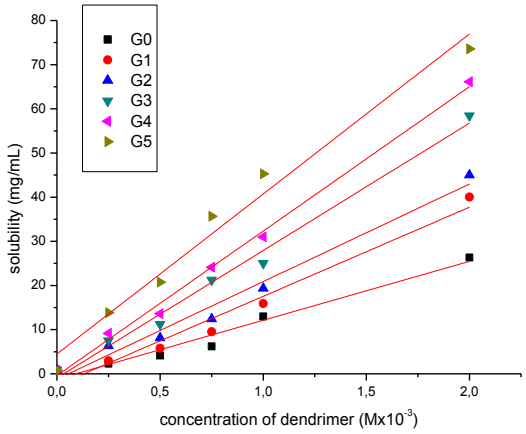
(NSAIDs) Solubility



(a)

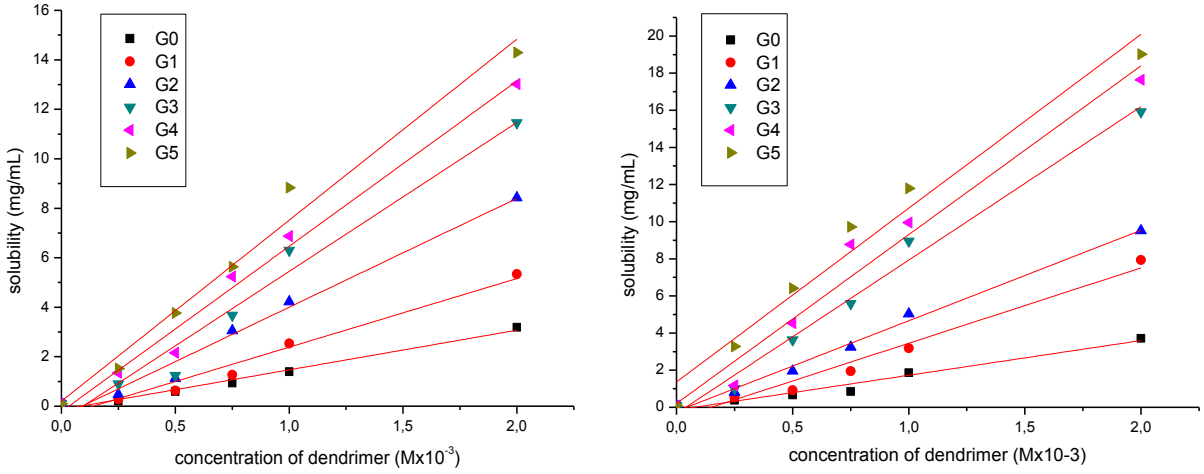


(b)

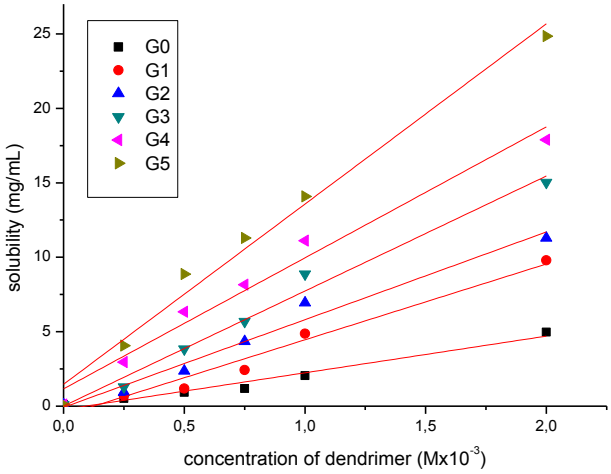


(c)

Figure 3.1 Ketoprofen solubility with **(a)**JAPD-440**, (b)**3000**, (c)**5000.

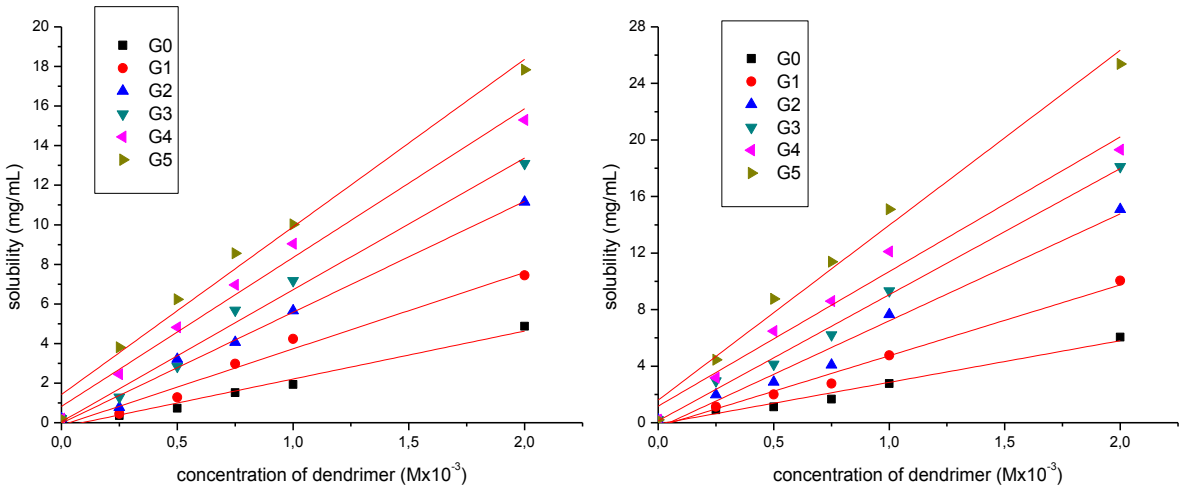


(a) **(b)**

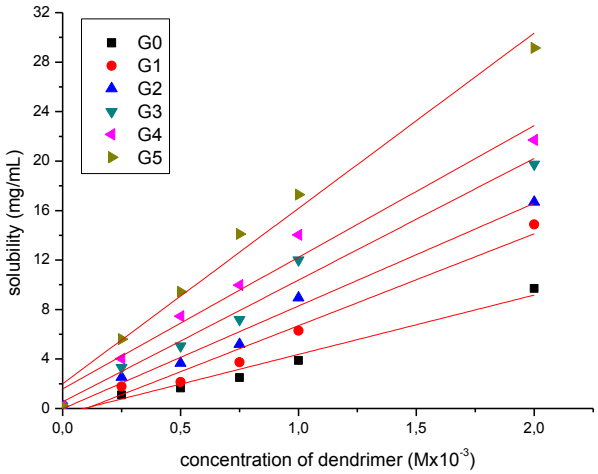


(c)

Figure 3.2. Ibuprofen solubility with **(a)**JAPD-440**, (b)**3000**, (c)**5000.

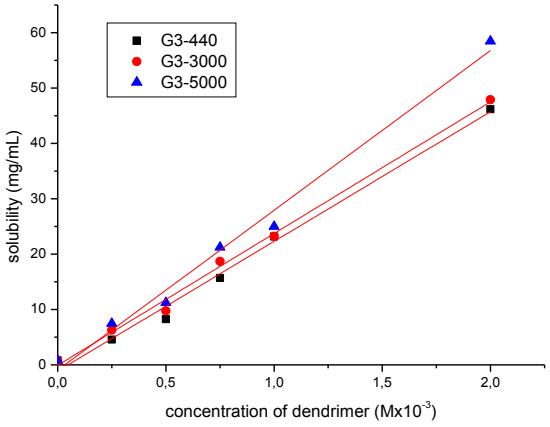


(a) **(b)**

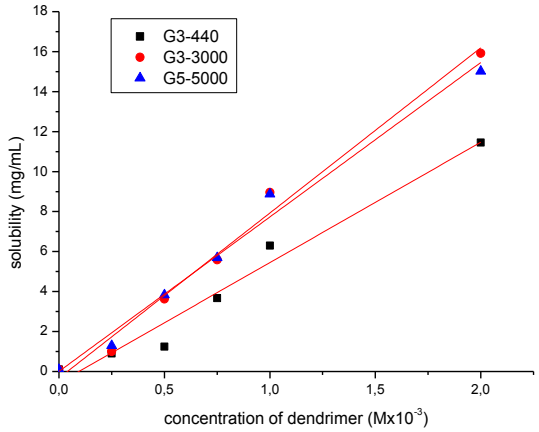


(c)

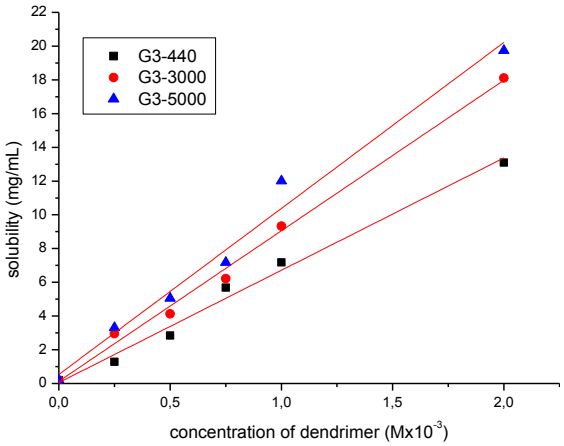
Figure 3.3. Diflunisal solubility with **(a)**JAPD-440**, (b)**3000**, (c)**5000.



(a)



(b)



(c)

Figure 3.4. The core effect (JAPD-G3) on Solubility of **(a)**Ketoprofen**, (b)** Ibuprofen**, (c)**Diflunisal.

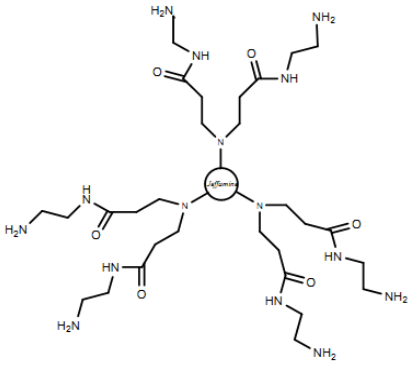
In recent years the research of dendrimers-drugs interactions is one of the most active area of pharmaceutical science. The interactions between dendrimers and drugs can be subdivided into the following three types: internal encapsulation (physical encapsulation, hydrophobic interaction and hydrogen-bond interaction); external electrostatic interaction and covalent conjugation [125-128].

In early studies, Milhem et al. investigated the potential of PAMAM dendrimers as drug cariers of hydrophobic drugs by ibuprofen [48]. They showed that PAMAM dendrimers could significantly enhance the solubility of ibuprofen compared to traditional surfactants such as sodium dodecyl sulphate. The approximate number of ibuprofen molecules attacted to each G4 PAMAM dendrimer was calculated to be 41. Kolhe et al. reported that the maximum number of ibuprofen associated with each G4 PAMAM dendrimer was 78 [49]. Since the number of primary amine groups on the surface of each G4 PAMAM was 64, it could be concluded that ibuprofen molecules can be either encapsulated in the interior cavities by hydrophobic interactions or attacted on the surface by electrostatic interactions. FT-IR and NMR results of the denrimer–drug complexes suggested that the carboxylate ion of ibuprofen predominantly formed a stable complex with the amine groups of cationic dendrimers [128].

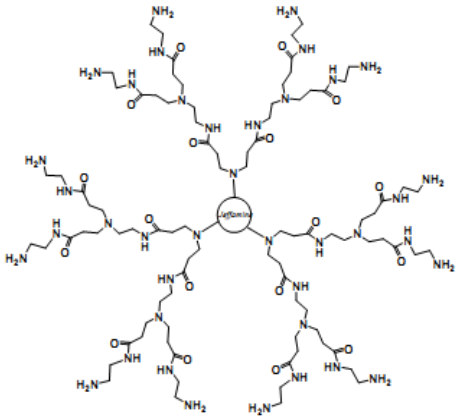
Previous studies suggested that the enhanced solubilities of insoluble drugs in cationic denrimer solutions were due to several interaction mechanisms between denrimers and drugs. First, the existence of a large number of relative nonpolar cavities in the interior of dendrimers provide denrimers the ability to encapsulate guests in cores by hydrophobic interactions [48,128].Second, high density of cationic functional groups on the surface of dendrimers, these dendritic architectures with the capability for electrostatic attachment of negatively charged guests [48,49].Third, tertiary amines and amide groups in internal cavities of denrimers can interact with spesific atoms (nitrogen or oxygen atoms) or functional groups (hydroxyl or caboxyl groups) of the guest molecules by hydrogen-bond formation [48].

Jeffamine cored PAMAM type dendrimers (JAPD) are different from ethylenediamine cored PAMAM dendrimers (EDAPD) due to structural properties. (figure 3.5).

(a)



(b)



(c)

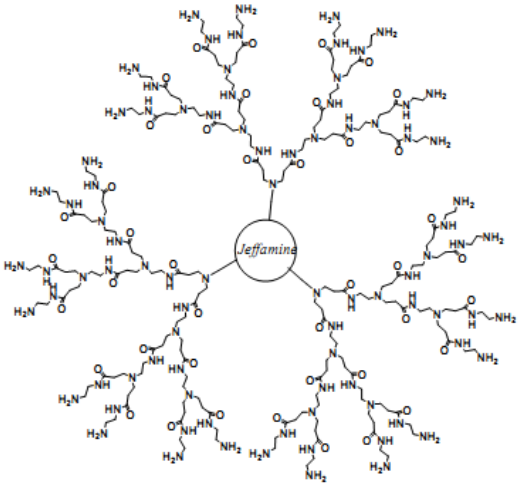


Figure 3.5. JAPD **(a)** G1, **(b)** G2, **(c)** G3 [129].

JAPD have more brancing units than EDAP, because of this, they have bigger caves and lots of external –NH2 groups. The number of primary amine groups on the surface of each G3,G4 and G5 JAPD was 24, 48 and 96, respectively.

In our study, the maximum number of ketoprofen associated with each G3, G4 and G5 JAPD were calculated 94, 119, 137; ibuprofen 42, 50, 57; diflunisal 37, 48, 60, respectively.

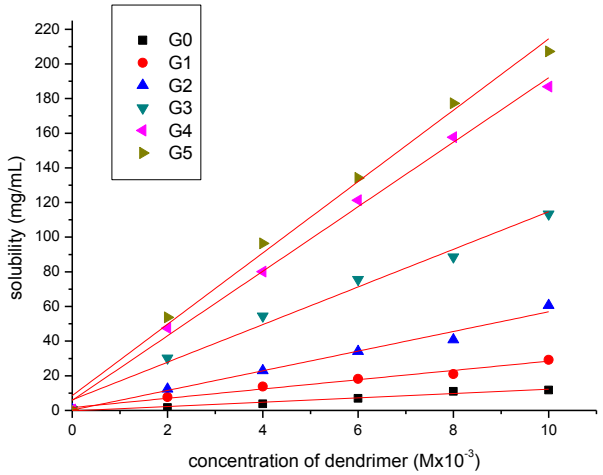
A series of solubility experiments were carried out to evaluate the effect of dendrimer concentration and generation on the solubility of model NSAIDs in the presence of Jeffamine cored PAMAM dendrimers. The results of the experiments were given in figure 3.1-3. The results showed that the solubility of NSAIDs has been significantly improved by dendrimers. The solubility of NSAIDs increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of Ketoprofen increased from 0.88 to 69.4 mg/ml; solubility Diflunisal increased from 0.22 to 25.37 mg/ml and Ibuprofen solubility increased from 0.12 to 19.06 mg/ml.

Above these ranges, the solubility of the NSAIDs was slightly lower getting lower due to the precipitation of an insoluble drug-dendrimer complexes. The similar solubility behavior was observed for the model drugs in all three Jeffamine cored PAMAM dendrimers. The solubility of NSAIDs is generally increased with the generation due to the effect of size and terminal groups on the solubility.

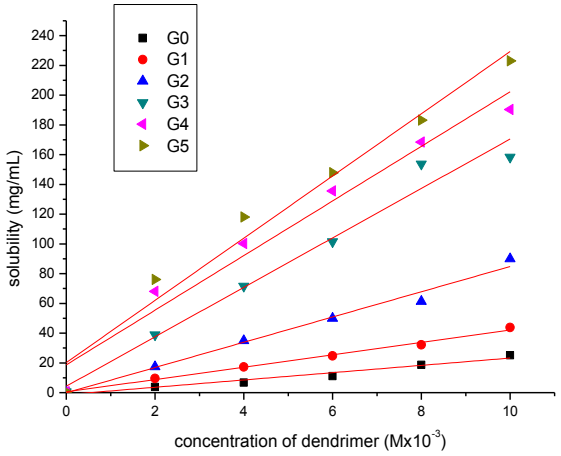
Possible mechanism of interactions between JAPD and NSAIDs: Drug molecules can be either attached high density of cationic functional groups on the surface of dendrimers by electrostatic interactions or encapsulated in the interior cavities by hydrophobic interactions.

The effect of core size of PAMAM dendrimers on the solubility of NSAIDs was investigated, the results are given in figure 3.4. The G-3 PAMAM dendrimers of Jeffamines used in this part to evaluate the effect of core size their MWs are 440, 3000 and 5000 g/mol. The results showed that the solubility of model drugs was increased in a certain amount with increasing core size.

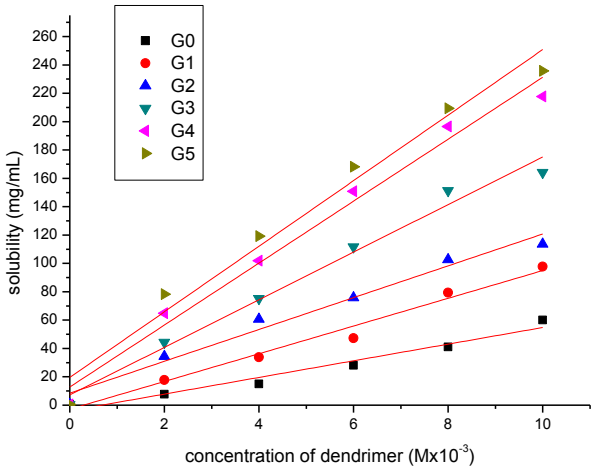
3.2.The Effect of Dendrimers and Core Size on Sulfamethoxazole (SMZ) Solubility



(a)



(b)



(c)

Figure 3.6. Sulfamethoxazole (SMZ) solubility with **(a)**JAPD-440**, (b)**3000**, (c)**5000.

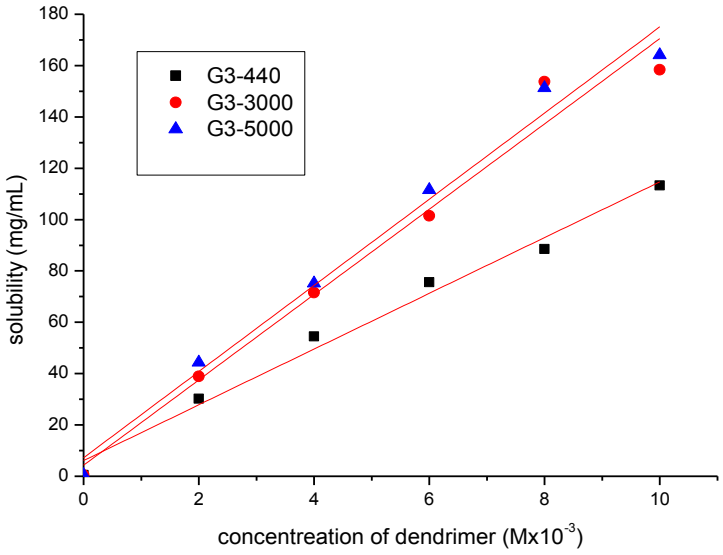


Figure 3.7. Effect of core size on solubility of SMZ in G3-JAPD

Due to specific properties such as internal cavities, terminal groups etc dendrimers are suitable for drug delivery systems. A series of solubility experiments were carried out to evaluate the effect of dendrimer concentration and generation on the solubility of SMZ in the presence of Jeffamine cored PAMAM dendrimers. The results of the experiments were given in figure 3.6. The results showed that the solubility of SMZ has been significantly improved by dendrimers. The solubility of SMZ increased linearly with increasing dendrimer concentration over the concentration range 0-10mM. Solubility of SMZ increased from 0.5 to 208 mg/ml (JAPD; core-440); from 0.5 to 233 mg/ml (JAPD; core-3000); from 0.5 to 289 mg/ml. The solubility of SMZ is generally increased with the generation due to the effect of size and terminal groups on the solubility.

The interactions between dendrimers and drugs can be subdivided into the following three types: internal encapsulation (involving physical encapsulation, hydrophobic interaction and hydrogen-bond interaction), external electrostatic interaction and covalent conjugation [125-128].

In Cheng et al. work [129], the aqueous solubility of sulfamethoxazole significantly increased with the help of PAMAM dendrimers. In dendrimer solutions, the acidic sulfamolyl group (-SO_2NH-) in sulfamethoxazole molecule generates a

negatively charged form which can be attached to the surface of dendrimers via electrostatic interaction. The cationic dendrimers significantly increased the solubilities of sulfamethoxazole. The results suggest that external electrostatic interaction contributes more to the solubility enhancement of SMZ than internal encapsulation.

3.3. *In vitro* Release of SMZ

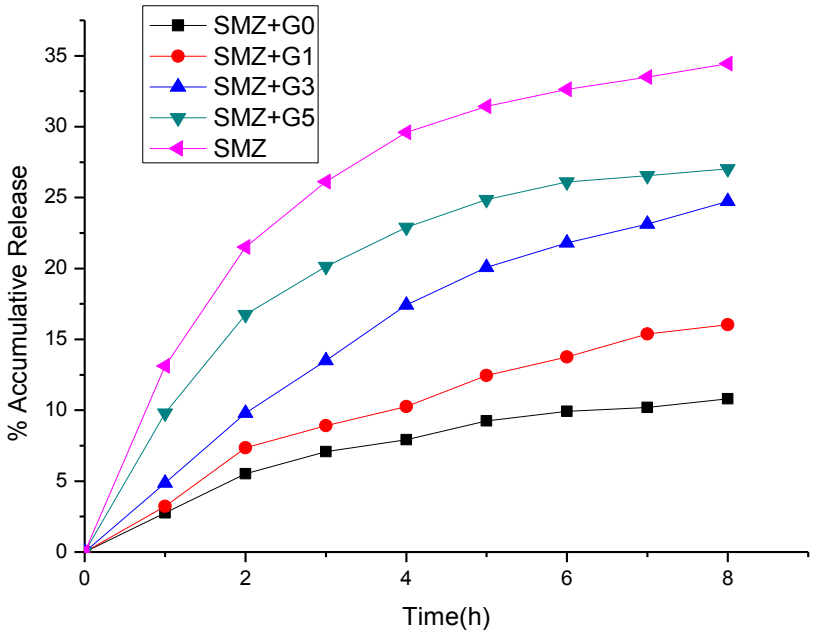


Figure 3.8. *In vitro* Release of SMZ in G0; G1; G3; G5 JAPD-3000 compared with the pure SMZ release behaviour

The results of *in vitro* release experiments are shown in figure 3.8. For example; after 1 hour, 13 % of the pure SMZ release only 4.8 % release from the SMZ-G3.0 dendrimer solution. And after eigth hours 35 % of pure SMZ release, 23 % release from the SMZ-G3.0 dendrimer solution.

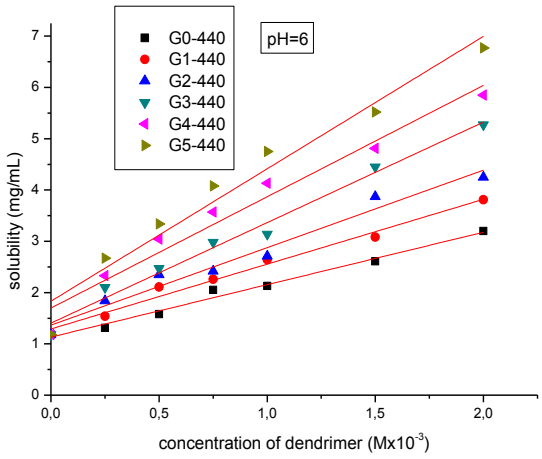
However, release rate increase with increasing dendrimer generation. In low generations (G0-3),electrostatic interaction more effective than encapsulation. Whereas, in high generation G4-5), hydrophobic binding more effective than electrostatic interactions. And also, hydrophobic caves in G5.0 dendrimer are larger than in G3.0 dendrimer. Release rate in G5 is close to pure SMZ release rate. In order to hydrophobic interactions are weak SMZ molecules release easily. But, in lower generation

hydrophobic caves are smaller than in higher generation. Because of this, release of drug is slower compared with in higher generations. Change of release rate is almost linear in G3. This result is shown that G3 is most suitable generation for controlled drug release.

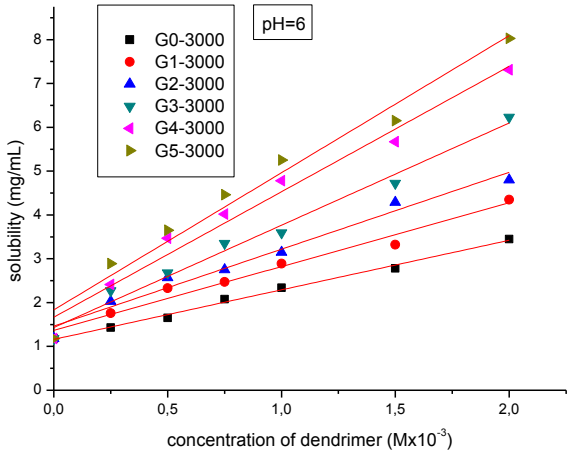
3.4.Antibacterial Activity of SMZ

In the experiments MIC for pure SMZ was 2 mg/ml while MIC for SMZ 1mM PAMAM complex was 0.25 mg/ml. This result indicated that 1mM concentration of PAMAM increased the antibacterial activity of SMZ against E.coli more than eight fold. This experiments were done by Nurdan Bülbül from the Department of Genetics and Bioengineering.

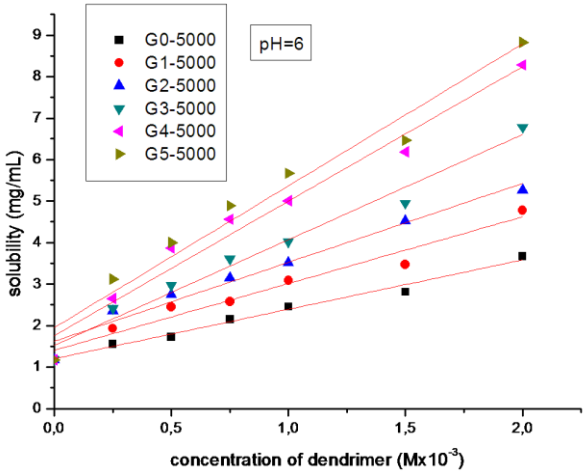
3.5. The Effect of Dendrimers and Core Size on Naproxen and L-Histidine Solubility at Different pH



(a)

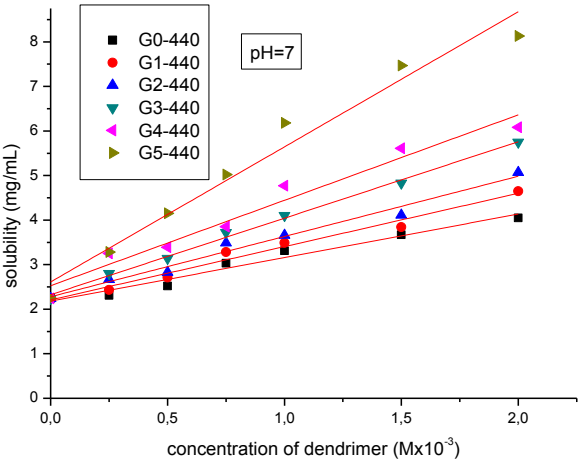


(b)

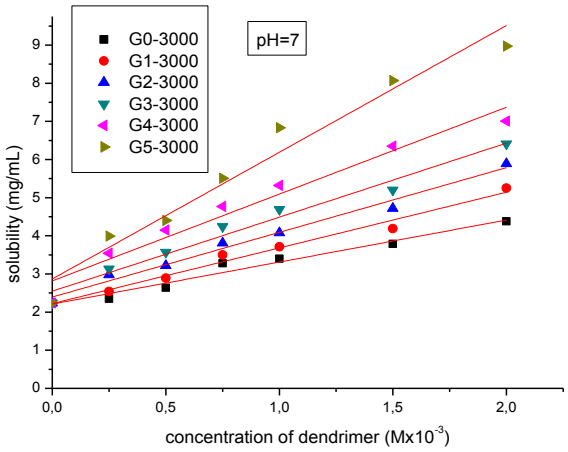


(c)

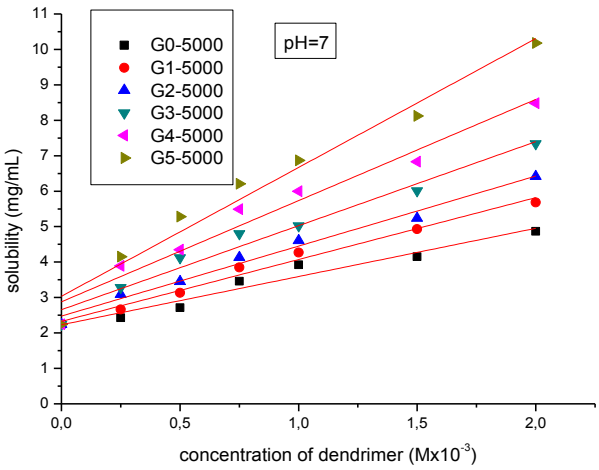
Figure 3.9. Naproxen solubility in pH=6 with **(a)**JAPD-440, **(b)**3000, **(c)**5000.



(a)

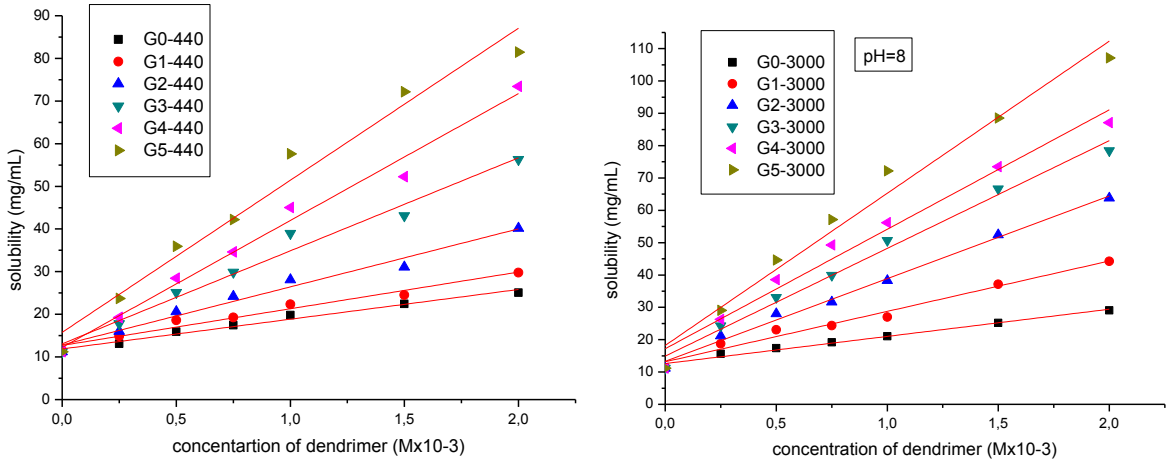


(b)

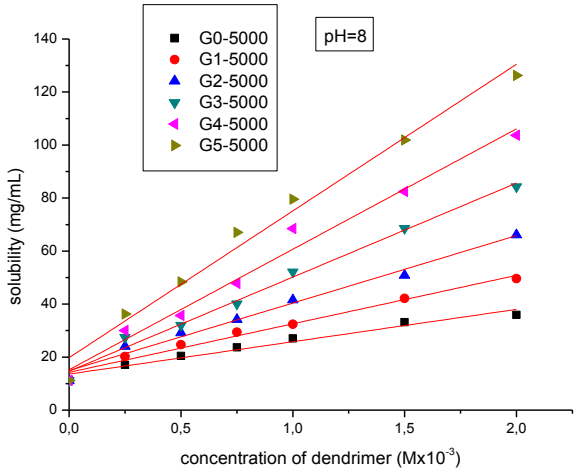


(c)

Figure 3.10. Naproxen solubility in pH=7 with **(a)**JAPD-440**,(b)**3000**, (c)**5000.



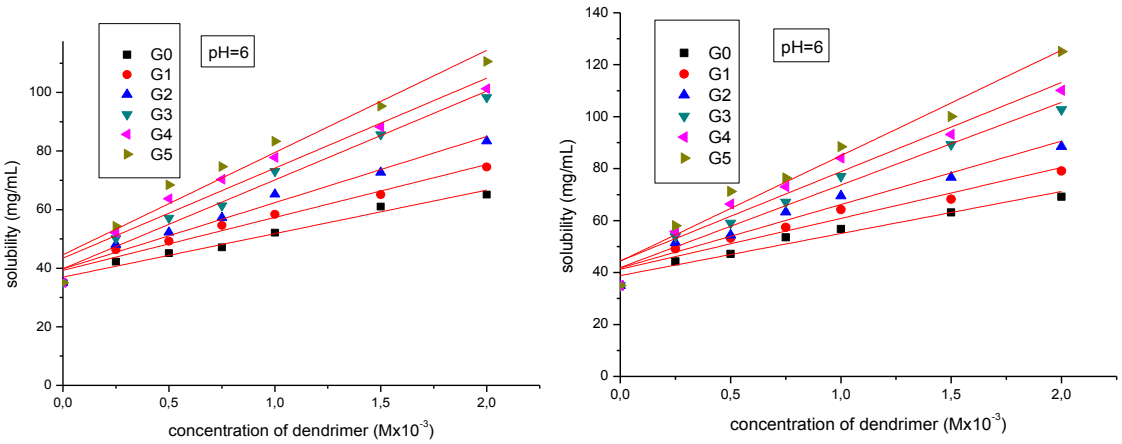
(a) **(b)**



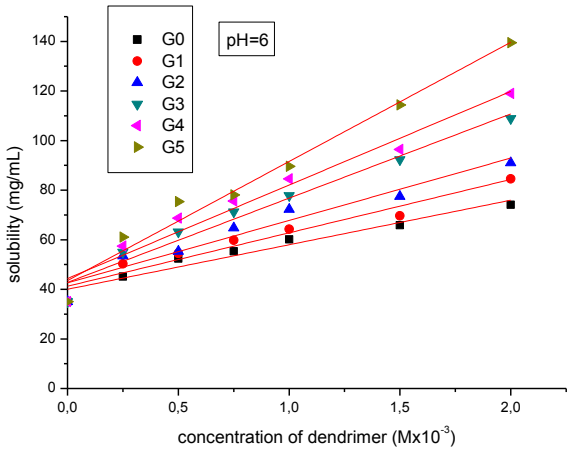
(c)

Figure 3.11. Naproxen solubility in pH=8 with

(a) JAPD-440**, (b)**3000**, (c)**5000.



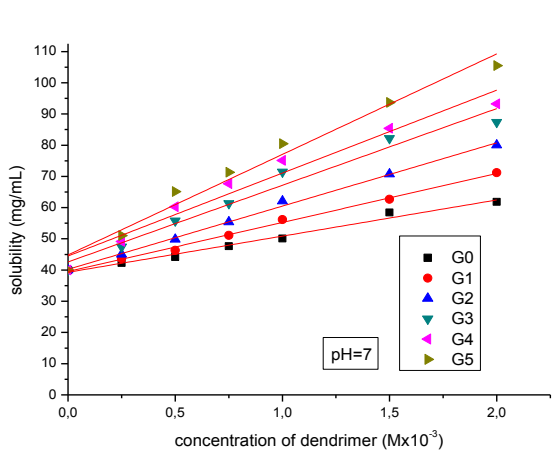
(a) **(b)**



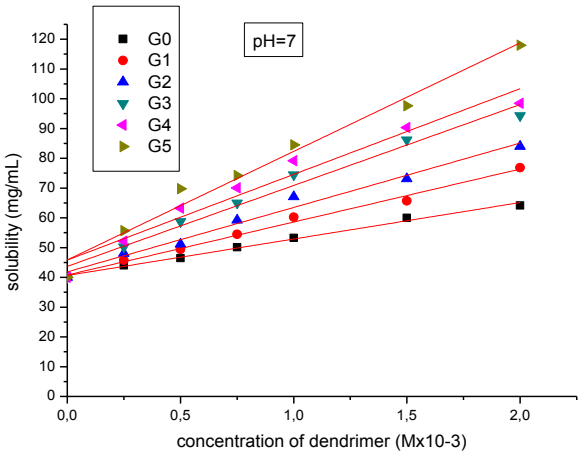
(c)

Figure 3.12. L-Histidine solubility in pH=6 with

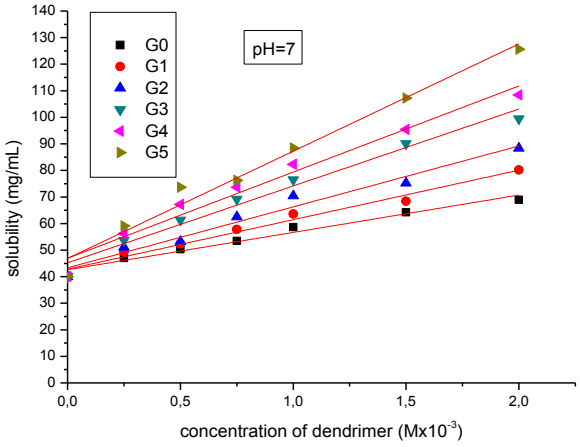
(a) JAPD-440 **(b)**3000 **(c)**5000.



(a)



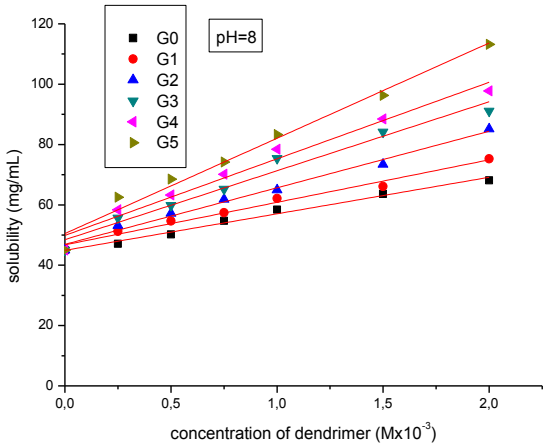
(b)



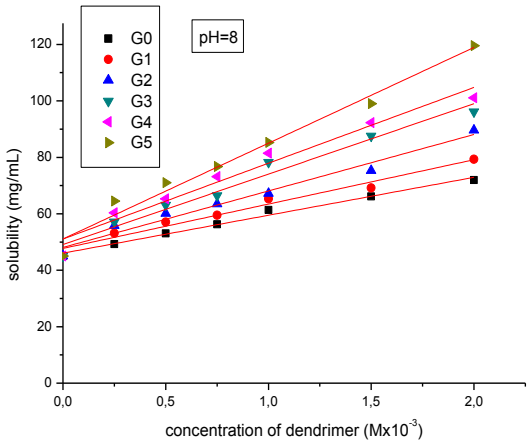
(c)

Figure 3.13. L-Histidine solubility in pH=7 with

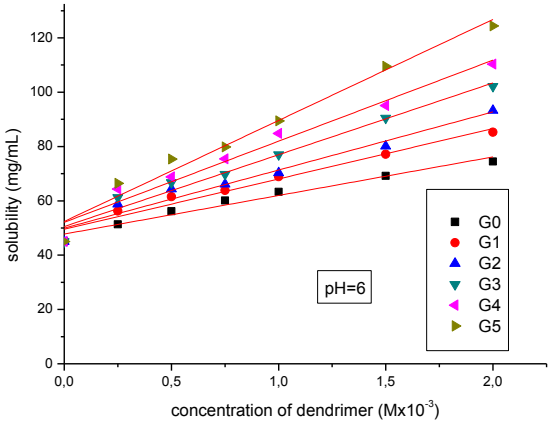
(a) JAPD- 440, **(b)**3000 **(c)**5000.



(a)



(b)



(c)

Figure 3.14. L-Histidine solubility in pH=8 with

(a) JAPD-440,**(b)** 3000, **(c)**5000.

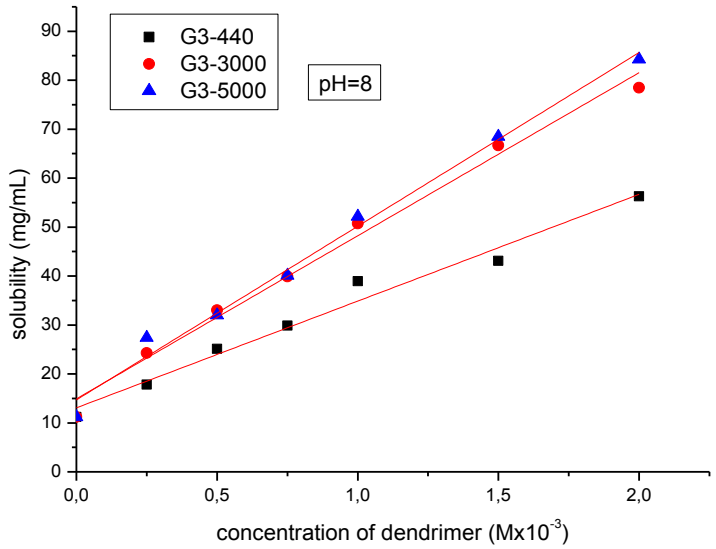


Figure 3.15. Effect of core size on solubility of Naproxen in G3-JAPD

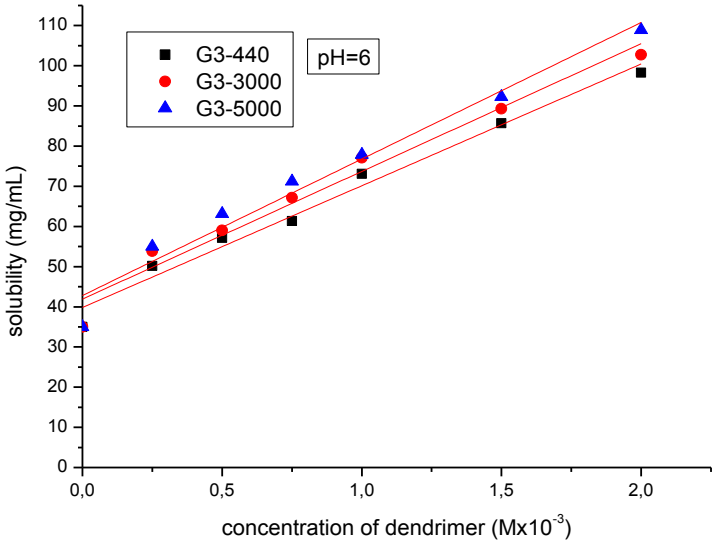


Figure 3.16. Effect of core size on solubility of L-Histidine in G3-JAPD

Naproxen is a weakly acidic NSAI drug. We measured its solubility as 1.2 mg/ml at pH=6; 2.2 mg/ ml at pH=7 and 11.2 mg/ml at pH=8, respectively.

L-Histidine is weakly basic an aminoacid. We measured its solubility was 45mg/mL at pH=6; 40 mg/mL at pH=7 and 35mg/mL at pH=8, respectively.

A series of solubility experiments were carried out to evaluate the effect of dendrimer concentration and generation on the solubility of Naproxen and L-Histidine at three different pH in the presence of Jeffamine cored PAMAM dendrimers. The results showed that the solubility of Naproxen has been significantly improved by dendrimers at each pH.

The solubility of naproxen increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of naproxen increased from 1.2 mg/mL to 8.83 mg/ml (pH=6); from 2.2 mg/mL to 10.18 mg/ml (pH=7); from 11.2 mg/mL to 126.24 mg/ml (pH=8). The highest solubility of naproxen is observed at pH=8. It may be due to its weakly acidic chacteristic.

The solubility of L-Histidine increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of L-histidine increased from 45 mg/mL to 139.47 mg/ml (pH=6); from 40 mg/mL to 125.65 mg/ml (pH=7); from 35 mg/mL to 124.37 mg/ml (pH=8). The highest solubility of L-histidine is observed at pH=6. It may be due to its weakly basic chacteristic.

The effect of core size of PAMAM dendrimers on the solubility of Naproxen and L-Histidine was investigated, the results are given in figure 3.15 and 3.16, respectively. The G-3 PAMAM dendrimers of Jeffamines used in this part to evaluate the effect of core size their MWs are 440, 3000 and 5000 g/mol. The results showed that the solubility of model drugs was increased in a certain amount with increasing core size.

CHAPTER 4

CONCLUSION

In this study, the effect of Jeffamine® cored PAMAM type dendrimers have been investigated on solubility of hyrophobic model drugs. In addition, in vitro release and antibacterial activity tests were done for a model drug. Solubility experiments were done with different generations, concentrations and core sizes of dendrimer.

First group model drugs were Non-Steroidal anti-inflammatory drugs (Ketoprofen, Ibuprofen and Diflunisal). The results showed that the solubility of NSAIDs has been significantly improved with dendrimers. The solubility of NSAIDs increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of Ketoprofen increased from 0.88 to 69.4 mg/ml; solubility of diflunisal increased from 0.22 to 25.37 mg/ml and ibuprofen solubility increased from 0.12 to 19.06 mg/ml, respectively. These experiments were done at constant pH=7.4. The G-3 PAMAM dendrimers of Jeffamines were used to investigate the effect of core size. Their MWs are 440, 3000 and 5000 g/mol. The results showed that the solubility of model drugs was increased in a certain amount with increasing core size.

Second drug was Sulfamethoxazole (SMZ). The results showed that the solubility of SMZ has been significantly improved by dendrimers. The solubility of SMZ increased linearly with increasing dendrimer concentration over the concentration range 0-10mM. Solubility of SMZ increased from 0.5 to 208 mg/ml (JAPD; core-440); from 0.5 to 233 mg/ml (JAPD; core-3000); from 0.5 to 289 mg/ml, respectively. Dendrimers were dissolved in water in these experiments. The results of *in vitro* release experiments for SMZ showed that all release rates were lower than pure SMZ release rate. However, release rate increase with increasing dendrimer generation. The rate is almost linear in G3. This result is shown that G3 is most suitable generation for controlled drug release. In the experiments MIC for pure SMZ was 2 mg/ml while MIC for SMZ 1mM PAMAM complex was 0.25 mg/ml. This result indicated that 1mM

concentration of PAMAM increased the antibacterial activity of SMZ against E.coli more than eight fold.

Last and thirth group drugs were Naproxen and L-Histidine. Naproxen was weakly acidic drug and L-Histidine was weakly basic drug. In this part, effect of dendrimers were investigated on solubility at different pH. The solubility of naproxen increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of naproxen increased from 1.2 mg/mL to 8.83 mg/ml (pH=6); from 2.2 mg/mL to 10.18 mg/ml (pH=7); from 11.2 mg/mL to 126.24 mg/ml (pH=8), respectively. The highest solubility of naproxen is observed at pH=8. The solubility of L-Histidine increased linearly with increasing dendrimer concentration over the concentration range 0-2 mM. Solubility of L-histidine increased from 45 mg/mL to 139.47 mg/ml (pH=6); from 40 mg/mL to 125.65 mg/ml (pH=7); from 35 mg/mL to 124.37 mg/ml (pH=8). The highest solubility of L-histidine is observed at pH=6.

All results showed that Jeffamine® cored PAMAM dendrimers have the potential to significantly increase the solubility of model drugs. In addition, higher solubility was measured than solubility of same drugs for ethylenediamine cored PAMAM dendrimers in literature. Also, release rate decreased compared with pure drug. This result is suitable for controlled drug release study. Under optimized conditions Jeffamine cored PAMAM dendrimers can be highly effective used as potential drug carriers for hydrophobic drugs.

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