MOLECULAR MODELING OF HYALURONIC ACID CONTANING CISPLATIN-LOADED POLYLAGTIC-CO-GLYCOLIC ACID BIOCONJUGATES FOR TARGETED DELIVERY TO CANCER CELLS

by

Abdulloh Madthing

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Dissertation report submitted in partial fulfillment of

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Bachelor of Engineering (Hons)
(Chemical Engineering)

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Universiti Teknologi PETRONAS

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Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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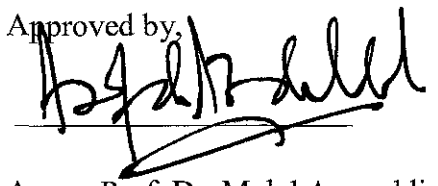
Approved by,



Assoc. Prof. Dr. Mohd Azmuddin Abdullah

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

September 2011

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

ABDULLOH MADTHING

ABSTRACT

This project will present the molecular modeling of hyaluronic acid (HA), cisplatin, polylactic-co-glycolic acid (PLGA) and PEG-bis amine as uni-molecular modeling and also conjugated forms of multi-molecular modeling for targeted delivery to cancer cells in 3D format by using Discovery Studios 2.5 software from Accelrys Inc. USA. Colorectal cancer cell will be the case study for targeted drug delivery. The methodologies to be used include molecular modeling design are molecular modeling, model development of cisplatin, PLGA, and HA. The simulation studies on drug delivery to colorectal cancer cells of the bioconjugated forms of the compounds will also be carried on.

ACKHOWLEDGEMENT

I am heartily thankful to my supervisor, Assoc. Prof. Dr. Mohd Azmuddin Abdullah, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject until the final stage of FYP process. To my university, Universiti Teknologi PETRONAS for giving me the opportunity to study as well as provide me with the full support in the material, equipment and laboratory.

Lastly, I offer my regards and blessings to my family and all of those who supported me in any respect during the completion of the project.

Thank you

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND OF STUDY

Cancer is one of the major disease that causes 13% of all human deaths in 2007 (Gul-e-Saba, et al., 2010). Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues and sometimes spreading to other locations in the body via lymph or blood (Anand et al., 2008)(Figure 1.1).

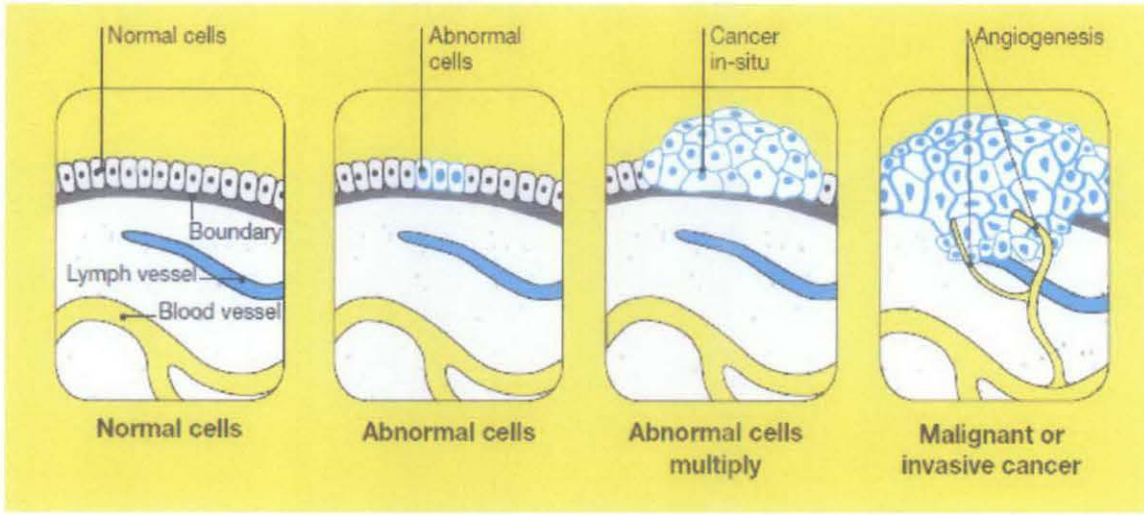


Figure1.1 How cancer starts (Cancer Council, 2011)

Chemotherapy is standard treatment of most disseminated cancers, but dose-limiting toxicities and the emergence of cancer cells resistant to chemotherapeutic drugs often reduce the clinical benefit (Chen et al., 2007). Cisplatin is one of the most effective chemotherapeutic agents against many forms of cancer including testicular, bladder, head and neck, ovarian, breast, lung, prostate, and refractory non-Hodgkin's lymphomas (Jamieson & Lippard, 1999; Rosenberg et al., 1969). Indeed, cisplatin is used to treat 50% of all cancers (Galanski et al., 2005), and it exerts its antitumor

effects by disrupting DNA structure in cell nuclei through the formation of intrastrand and interstrand cross-links (Wang & Lippard, 2005).

The development of drug delivery systems has improved the therapeutic and toxicological properties of existing chemotherapies and facilitated the implementation of new ones. By including the drug in technologically optimized drug delivery systems or conjugating the drugs with different polymers, it is possible to modify the pharmacokinetics and biodistribution of the drugs improving the efficacy and security of the therapy (Peppas & Blanchette, 2004).

Nanoparticulate delivery systems, such as those based on poly (lactic-co-glycolic acid) (PLGA) polymers, have been studied extensively for many years. PLGA polymers have the advantage of being well characterized and already commercially used for microparticulate drug delivery systems (Allemann & Leroux, 1999) PLGA polymers are biocompatible, biodegradable, and bio-resorbable (Wise et al., 1979). Natural and synthetic polymers including albumin, fibrinogen, alginate, chitosan, and collagen have been used for the fabrication of nanoparticles. However, among all of these, lactic-glycolic acid copolymers are the most frequently employed materials due to their biocompatibility and biodegradability (Orive et al., 2005)

Hyaluronic acid (HA) is a glycosaminoglycan found distributed throughout the connective, epithelial and neural tissues (Laurent et al., 1995). It is one of the main components of the extracellular matrix, contributes significantly to cell proliferation and migration and is also involved in the progression of some malignant tumors where it is highly concentrated. Besides, it turns out to be an important signal for activating kinase pathways (Misra et al., 2006; Ohno et al., 2006) and regulating angiogenesis (Rooney et al., 1995). The physiological function of HA varies greatly depending on HA size, and the presence or absence of HA binding proteins and cell surface receptors (Tammi et al., 2002; Toole, 2004). Many tumors are enriched in HA (Tammi et al., 2002; Boregowda et al., 2006). HA levels can be increased within the tumor cells themselves or within the tumoral stroma (Anttila et al., 2000; Auvinen et al., 2000).

The advanced tool that could help drug developers are in the use of computational modelling for designing, testing and simulating the molecular structure of the drugs. Drug modelling helps to better understand the interactions of drug with microenvironment in the body at the disease sites. In this study, hyaluronic acid contaning cisplatin-loaded polylagtic-co-glycolic acid bioconjugates as a potential anticancer drug will be modelled and simulated for targeted delivery to cancer cells.

1.2 PROBLEM STATEMENT

Designing the molecular structure of the vehicle contains drug by computational approach could be the best solution as it provides better understanding of effective delivery, drug accumulation in cancer cells, reduce the experimental cost , trial failure and save time.

1.3 OBJECTIVES

1. To model 3-D Uni-Molecular modeling of Hyaluronic Acid (HA), Cisplatin , Polylactic-co-glycolic acid and Multi-molecular modeling of Cisplatin, PLGA, PEG, HA and conjugated molecular 3D Structures PLGA conjugated HA ,cisplatin conjugated HA and PLGA-PEG-HA conjugates. Detail Inter and Intra-molecular measurement of Bond length with bond order and type of bond were also discussed.
2. To simulate the controlled, targeted drug delivery system of the Hyaluronic Acid (HA) containing Cisplatin-loaded PLGA nanosphere bioconjugates.

1.4 SCOPE OF STUDY

The project will cover molecular modeling of Hyaluronic Acid (HA) containing Cisplatin-loaded PLGA nanosphere bioconjugates as an anti-cancer drug for controlled, targeted delivery to cancer cells. The Discovery Studio 2.5 (Accelrys Inc., USA) will be used as a tool to design the molecules and simulate the delivery to targeted site.

CHAPTER 2

LITERATURE REVIEW

2.1 COLORECTAL CANCER

Colorectal cancer is cancer that starts in the colon or the rectum. These cancers can also be referred to separately as colon cancer or rectal cancer, depending on where they start. The colon and rectum together make up the large intestine, part of the body's digestive system. The colon is a large muscular tube (approximately five feet long) that collects and stores waste which then passes into the rectum. Tumors can develop within the walls of the colon and/or rectum tissue which are called polyps (American Cancer Society, 2005) (Figure 2.1).

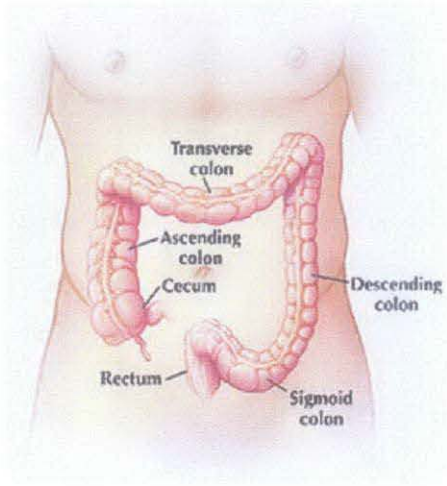


Figure 2.1 Colorectal in human body (www.fitcare.net, 2009)

Colorectal cancer is one of the most common tumors and a major cause of cancer death worldwide (Fiore et al., 2007). Recent studies from the Cancer incidence in five continents volume IX have shown an increasing incidence of colorectal cancer in Asian populations. According to the Second Report of the National Cancer Registry, colorectal cancers accounted for 14.2% of male cancers and 10.1% of female cancers in Malaysia, making it the commonest cancers among men and the third most common cancer among women respectively. The incidence of colon cancer in Chinese was 2 times higher compared to other ethnic groups (Figure 2.2).

Colorectal cancer is the third commonest cause of cancer-related mortality in Malaysia.

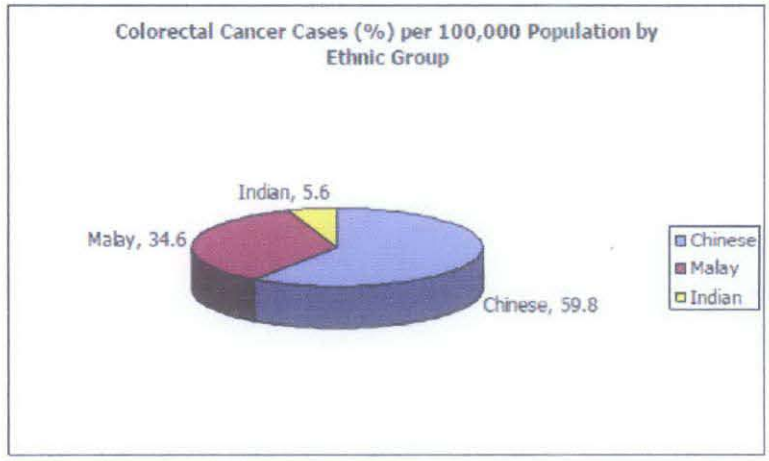


Figure 2.2 Colorectal Cancer Cases (%) per 100,000 Populations by Ethnic Group (Norsidawati, 2009)

These tumors can either be malignant (cancerous) or benign. Benign polyps may develop into adenomatous polyps over time and infact, about 85% of all colorectal cancers develop fromadenomatous polyps (American Cancer Society, 2005). There are 5 stages in the development of colorectal cancer (Figure 2.3).

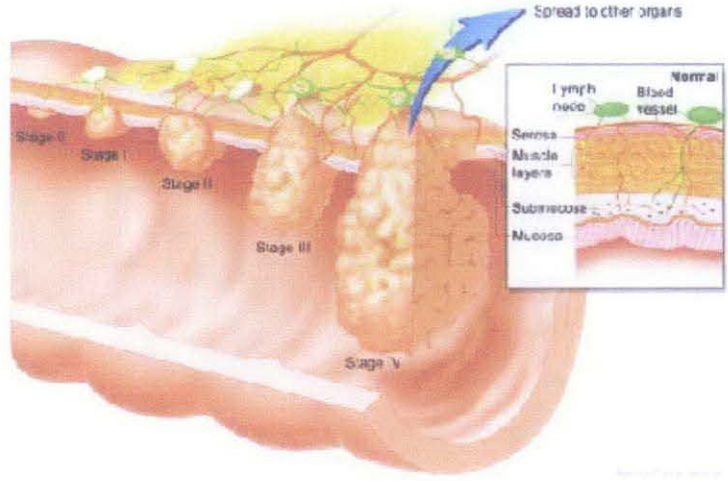


Figure 2.3 Colorectal Cancer stages (National Cancer Institute)

Stage 0: this is the earliest stage, the cancer is found only in the innermost of the colon

Stage I: the cancer starts to evolves in the inner wall of the colon

Stage II: the cancer become bigger and spread outside the colon but not yet to the lymph nodes (Lymph nodes are small, bean-shaped structures that are part of the body's immune system.)

Stage III: the cancer spreads nearby the lymph nodes but not to the other parts of the body

Stage IV: the cancer has spread to the other part of the body such liver and lungs, this is the most serious stage of the colorectal cancer (Adapted from National Cancer Institute).

Several types of cancer can start in the colon or rectum. More than 95% of colorectal cancers are a type of cancer known as **Adenocarcinomas**. These cancers start in cells that form glands that make mucus to lubricate the inside of the colon and rectum. Other, less common types of tumors may also start in the colon and rectum. These include **Carcinoid tumors.** These tumors start from specialized hormone-producing cells in the intestine. **Gastrointestinal stromal tumors (GISTs)** start from specialized cells in the wall of the colon called the interstitial cells of Cajal. Some are benign (noncancerous); others are malignant (cancerous). These tumors can be found anywhere in the digestive tract, but they are unusual in the colon. **Lymphomas** are cancers of immune system cells that typically start in lymph nodes, but they may also start in the colon, rectum, or other organs. **Sarcomas** can start in blood vessels as well as in muscle and connective tissue in the wall of the colon and rectum. Sarcomas of the colon or rectum are rare. (Adapted from American cancer society)

2.2 CISPLATIN AS ANTI-CANCER DRUG

The biological activity of cisplatin, was discovered serendipitously about 125 years after the initial report of its synthesis and characterization. Although the synthesis and characterization of cisplatin was first reported by Peyrone in 1845 (Peyrone, 1845), its anticancer properties remained unnoticed until the mid-1960s, when

Rosenberg and co-workers studied the effects of electric fields on Escherichia coli growth (Rosenberg et al., 1965). The great impact in the treatment of cancer of the platinum coordination complex cisplatin, [*cis*-diamminedichloro platinum(II)] or *cis*-DDP, is a paradigm within the use of metals in medicine. In fact, cisplatin and its analogue carboplatin, [*cis*-diammine-1,1-cyclobutanedicarboxylate platinum(II)] are among the most commonly used antitumor drugs today Tumor resistance to *cis*-DDP coupled with cisplatin toxicity have stimulated the search for other antitumor-active platinum complexes with improved pharmacological properties. The only registered platinum drug that has consistently demonstrated antitumor activity against cisplatin resistant tumors such as colorectal cancers is oxaliplatin, [*trans*-L-1,2-diaminocyclohexaneoxalatoplatinum(II)] (Cvitkovic,1998). (Fig. 2.4 depicts the structures of the three platinum antitumor drugs currently approved for clinical use by both the American Food and Drug administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) as well as the structure of the inactive isomer of cisplatin, transplatin (*trans*-DDP)).

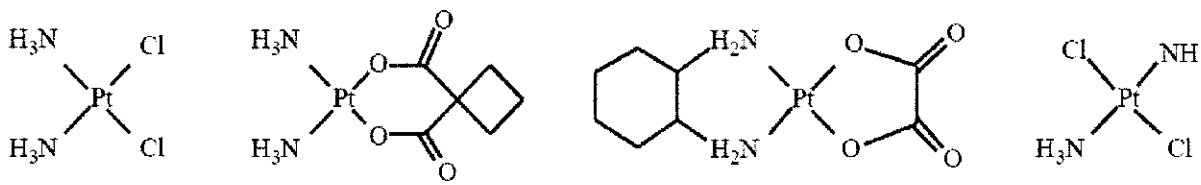


Figure 2.4 (A) cisplatin, (B) carboplatin, (C) oxaliplatin and (D) biologically inactive transplatin. (Cepeda et al., 2007))

The mechanism of anticancer activity involves formation of platinum– DNA adducts that are capable of inhibiting DNA and RNA synthesis (Johnson et al., 1989). and inducing programmed cell death (Barry et al.,1990; Ormerod et al., 1996). Cisplatin binds preferentially to the N7 position of purine residues. The monofunctional adduct subsequently closes to a bifunctional adduct by linking a second purine that can be either of the same strand or of the opposite strand (Sherman & Lippard, 1987). There is general consensus that the antitumor efficacy of cisplatin is

associated with the formation of DNA 1,2-intrastrand d(GpG) or d(ApG) cross-links (Lippert et all.,1984). The 1,2-intrastrand crosslinks locally unwind and bend doublestranded DNA toward the major groove (Takahara et al., 1995,; Takahara et al., 1996, Rice et al., 1988), and the disturbance of DNA secondary structure seems to be the ultimate reason for inhibition of DNA replication and/or transcription and for triggering apoptotic cell death (Sorenson et al., 1990; Barri et al., 1990).

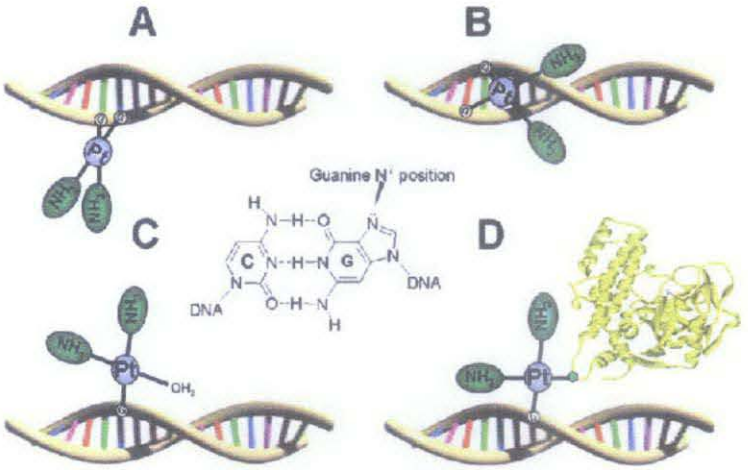


Figure 2.5 Main adducts formed after binding of *cis*-DDP to DNA. (A) 1,2-intrastrand cross-link, (B) interstrand cross-link, (C) monofunctional adduct, and (D) protein-DNA cross-link. The main site of attack of *cis*-DDP to DNA (N7 of guanine) is shown in the central panel. (Cepeda et al., 2007)

Cisplatin is one of the most potent anticancer agents available today and is widely used in the treatment of many malignancies, including testicular, ovarian, bladder, head and neck, small cell and non-small cell lung cancers (Comis, 1994) and (Boulikas & Vougiouka, 2004). However, its use is associated with severe side effects, such as acute nephrotoxicity and chronic neurotoxicity (Ponzani et al., 1994). A more selective administration (targeting) of cisplatin to cancer cells would reduce drug toxicity and enhance its therapeutic potential. Passive targeting of anticancer drugs to tumors could be achieved by attaching them to long circulating soluble or particulate carriers taking advantage of the enhanced permeability and retention (EPR) effect. The EPR effect is a result of leaky capillaries adjacent to solid tumors

and a lack of a lymphatic system for the drainage of drugs back to the systemic circulation (Kwon, 1998). The association of drugs with long-circulating carriers alters drug pharmacokinetics and results in increased drug accumulation in tumors, based on the EPR effect. For a more selective delivery to tumors, cisplatin has been administered in the form of soluble drug–polymer conjugates (Avichezer et al., 1998; Ferruti et al., 1999 Malik et al., 1999) and (Gianasi et al., 1999), or in the form of colloidal carriers, such as pegylated liposomes (Newman et al., 1999), poly(aspartic) acid-poly(ethylene glycol) micelles (Nishiyama et al., 2011), and poly(caprolactone)-poly(ethylene glycol) or poly(caprolactone)-poly[2-(N,N-dimethylamino)ethyl methacrylate] micelles (Van Kirk et al., 2006)(Figure 2.6 The example of design and construction of nanoparticles by PLGA-PEG polymer with the cisplatin-bearing polymer (PLA-cisplatin).

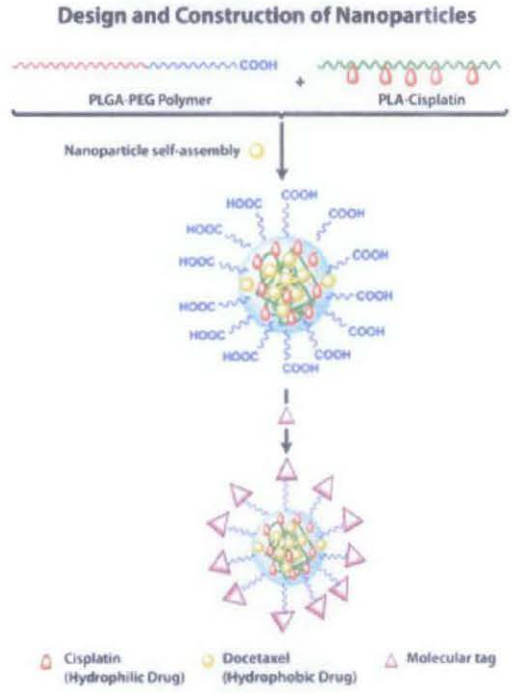


Figure 2.6 PLGA-PEG polymer with the cisplatin-bearing polymer (PLA-cisplatin). Docetaxel is then added to the mix. As nanoparticles self-assemble, docetaxel is encapsulated inside. In the final step, a molecular tag is added to enable the particle to navigate itself to the target of interest (PNAS, 2010)

2.3 PLGA AS A DRUG DELIVERY AGENT

The development of drug delivery systems has improved the therapeutic and toxicological properties of existing chemotherapies and facilitated the implementation of new ones. By including the drug in technologically optimized drug delivery systems or conjugating the drugs with different polymers, it is possible to modify the pharmacokinetics and biodistribution of the drugs improving the efficacy and security of the therapy (Peppas & Blanchette, 2004).

Recent developments in nanotechnology have allowed new research strategies to flourish in the field of drug delivery. There has been considerable interest in developing nanoparticles as effective drug delivery carriers (Allemann et al., 1993)(Figure 2.7).

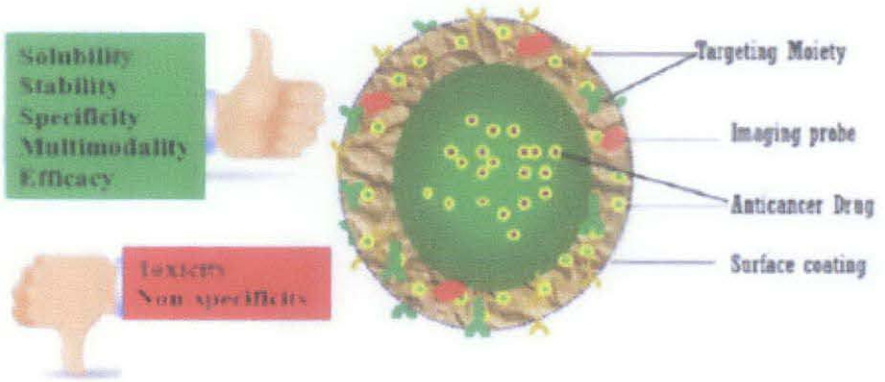


Figure 2.7 Advantage of Nanocarriers (Nanoparticles as nano-carriers can increase solubility, stability, specificity, multimodality, and efficacy, while reducing toxic side effects and improving upon the non-specificity of conventionally delivered cancer treatments) (Shaker et al., 2011).

A critical advantage in treating cancer with advanced, non-solution based therapies is the inherent leaky vasculature present serving cancerous tissues. The defective vascular architecture, created due to the rapid vascularization necessary to serve fast growing cancers, coupled with poor lymphatic drainage allows an enhanced permeation and retention effect (EPR effect) (Teicher, 2000; Sledge & Miller, 2003). Targeting the tumor vasculature is a strategy that can allow targeted delivery to a wide range of tumor types (Eatock et al., 2000; Reynolds et al., 2003).Tremendous

opportunities exist for using nanoparticles as controlled drug delivery systems for cancer treatment (Panyam & Labhasetwar, 2003; Birnham & Branno-Peppas, 2004). Natural and synthetic polymers including albumin, fibrinogen, alginate, chitosan, and collagen have been used for the fabrication of nanoparticles. However, among all of these, lactic-glycolic acid copolymers are the most frequently employed materials due to their biocompatibility and biodegradability (Orive et al., 2005).

Nanoparticulate delivery systems, such as those based on poly(lactic-co-glycolic acid) (PLGA)(Figure 2.8 Shows structure of PLGA) polymers, have been studied extensively for many years. PLGA polymers have the advantage of being well characterized and already commercially used for microparticulate drug delivery systems (Allemann et al., 1999). The PLGA polymer had several advantages like good mechanical properties, low immunogenicity and toxicity, excellent biocompatibility and predictable biodegradation kinetics. The wide acceptance of the lactide/glycolide polymers as suture materials made them attractive candidates for biomedical applications like ligament reconstruction, tracheal replacement, surgical dressings, vascular grafts and nerve, dental and fracture repairs (Lewis, 1990; Visscher et al., 1988). Polymeric nanoparticles of this polymer are used for the delivery of various drugs (antipsychotics, anesthetics, antibiotics, antiparasites, antitumorals, hormones, proteins, etc) (Verger et al., 1998). Drug carriers must show persistence in systemic circulation after intravenous administration in order to be useful for controlled drug delivery and/ or targeting applications. The biodistribution properties of polylactide-coglycolide- co-polyethylene glycol (PLGA-PEG) nanoparticles have been studied in experimental animals after labeling them with radioactive agents. The results showed a character of extending half-life and ability to control the release of the encapsulated compounds (Li et al., 2001). PLGA-PEG nanoparticles were found to exhibit linear, dose-independent pharmacokinetics for a dose range of 150–1050 μg per mouse. The dosage-independence of the PLGA-PEG nanoparticles would provide further advantages for their application in controlled drug delivery and targeting (Beletsi et al., 2005). PLGA nanoparticles can be formed by interfacial deposition following solvent displacement technique (Fessi et al., 1989). Several compounds such as indomethacin (Cauchetier et al., 2003) and muramyl dipeptide MDB-B305 (Barichello et al., 1999) have been incorporated in this tech-nique.

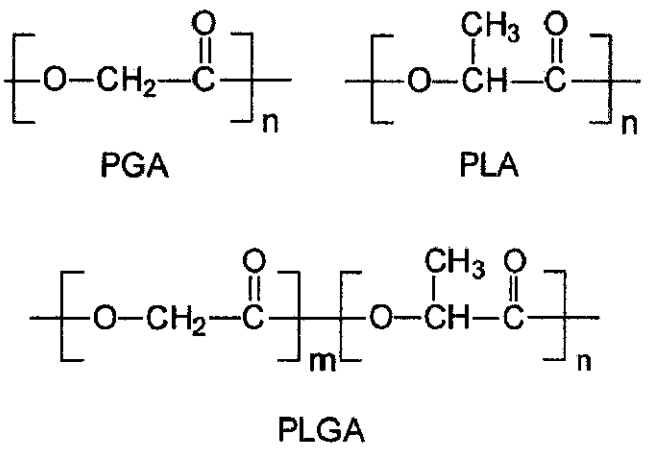


Figure 2.8 Chemical structure of poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and poly (lactic-co-glycolic acid) (PLGA) (Ikada et al., 2000)

The use of polyethylene glycol is one of the most common practices for passive targeting to the tumor site. A biocompatible and biodegradable nanoparticulate system using long circulating PLGA-monomethoxy-poly (polyethylene glycol) (PLGA-mPEG) nanoparticles has been synthesized (Avgoustakis et al., 2003). The main advantage of this nanoparticulate system, encapsulating cisplatin, is its potential for passive cancer targeting. Cisplatin-doped PLGA-mPEG nanoparticles showed an initial rapid release of drug, followed by a relatively slow release phase in vitro at a pH of 7.4, as shown by most PLGA-based nanoparticles. However, it was observed that the release kinetics depend on the ratio of PLGA to mPEG. The amount of released cisplatin in the initial burst increases with the increase of the amount of mPEG in the nanoformulation. Intravenous administration in BALB/c mice of this nanoformulation incorporating cisplatin resulted in an increased cisplatin residence time in the systemic circulation (Avgoustakis et al., 2003). The comparative cytotoxic effects of cisplatin were demonstrated when encapsulated in PLGA-mPEG nanoparticles on human prostate cancer LNCaP vs. free cisplatin (Gryparis et al., 2007) (Figure 2.9).

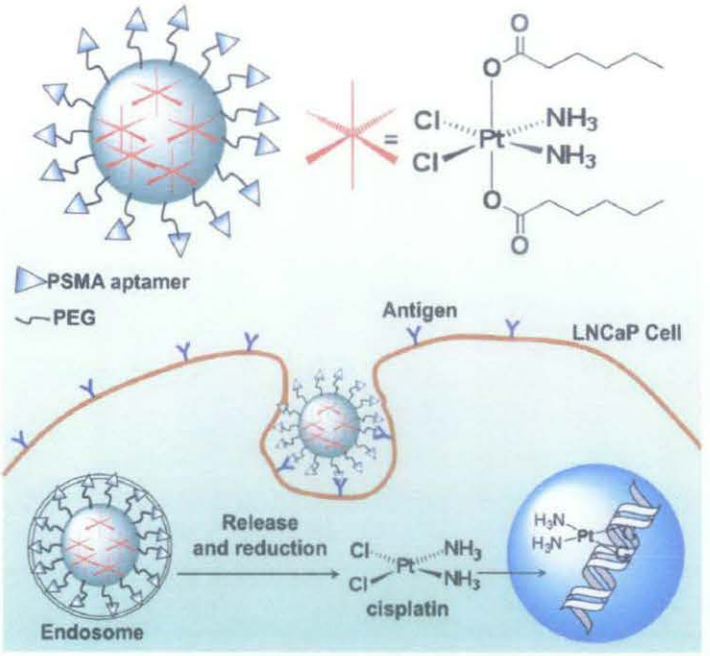


Figure 2.9 Representation of the Pt-PLGA-b-PEG-Apt-NP construct. Chemical structure of the Pt (IV) prodrug 1 and intracellular reduction for the release of active cisplatin in PSMA expressing human prostate cancer LNCaP cells after receptor mediated endocytosis of Pt-PLGA-b-PEG-Apt-NP (Dhar et al., 2011)

2.4 HYALURONIC-ACID CONTAINING BIOCONJUGATES

Hyaluronic acid (HA) is a repeating disaccharide units composed of D-glucuronic acid and N-acetyl-D-glucosamine and can be considered as the main component of the extracellular matrix of the cell membrane and plays an important role in the mechanical support of the cell of many tissues such as the skin, the tendons, the muscles and cartilage (Mohapatra, et al., 2008). (Fig 2.10 shows the structure of an HA).

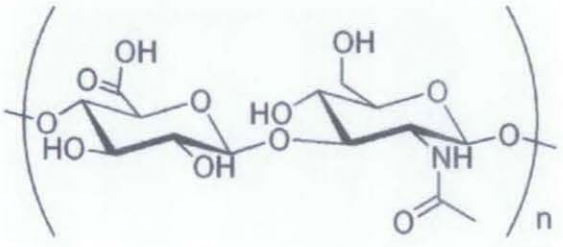


Figure 2.10 Structure of an HA (www.anikatherapeutics.com , access 2011)

HA can interact with CD44, its receptor at the cell surface which can lead to the site-specific delivery or targeted delivery of the drug (Yadav et al., 2010). This case on degrade inside the cells (Luo et al., 2009). The modeling development will include HA, which can be encapsulated and formulated with various peptide, PLGA and small molecular drugs (cisplatin) for cell specific drug delivery. The nanoparticles are basically from natural polymers, biocompatibles and biodegradables. The PLGA allow the controlled release of the active molecules they transport which is anti-cancer drug and their orientation towards the target tissues (Mohapatra, et al., 2008). Targeting of anti-cancer agents to tumor cells and tumor metastases can be accomplished by receptor-mediated uptake of bioconjugates of anti-cancer agents conjugated to HA, followed by the release of free drugs through the degradation of HA in cell compartments (Luo, et al., 2009)(Figure 2.11 Shows interaction of HA-drug with CD44 receptors on tumor cell) (Figure 2.12 Synthesis and release of HA-Pt conjugates).

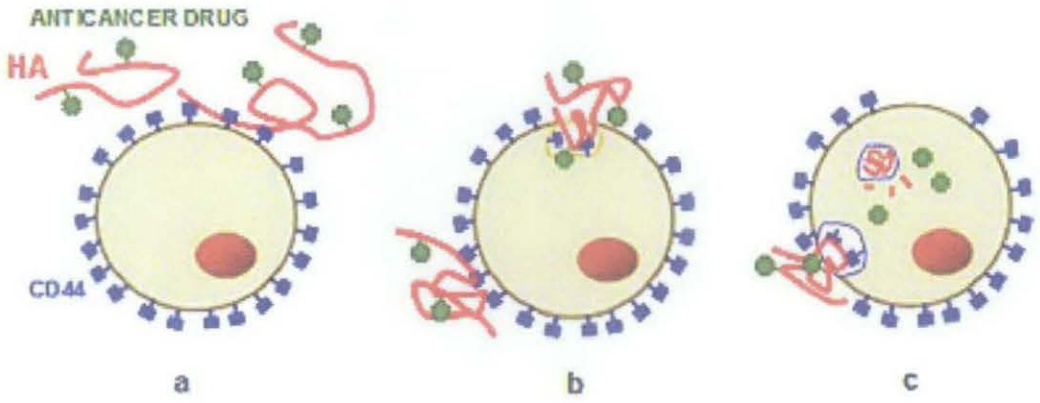


Figure 2.11 Interaction of HA-drug with CD44 receptors on tumour cell (a), cell absorbs molecule by engulfing it through the CD44 "door" (b), HA-drug degrades and drug is released directly into the key areas of the cell (e.g. lysosomes), causing it to die (c)(Di Meo et al., 2007,2008).

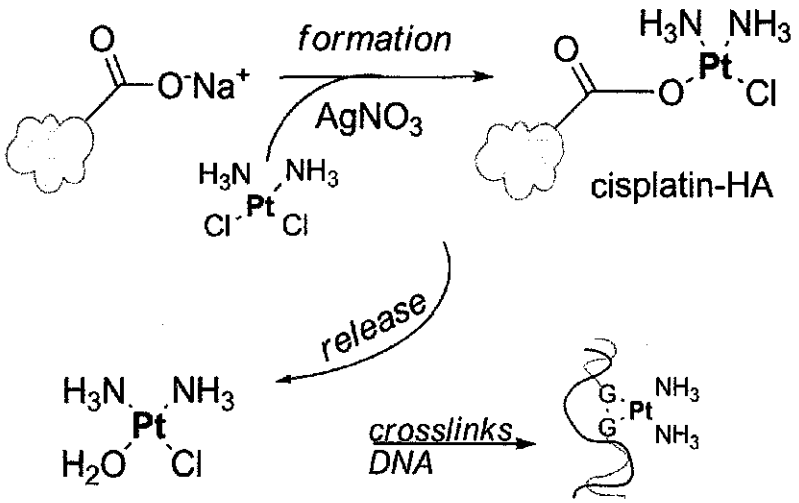


Figure 2.12 Synthesis and release of HA-Pt conjugates (Surg, 2009).

Hyaluronan or HA an important molecule (glycosaminoglycan) found almost everywhere in the human body shows a selective interaction with a specific cellular receptor, the CD44, which is over-expressed in many cancers. By the linkage of an anticancer drug on the HA chain, a more selective target and release of the chemotherapeutic agent to tumour cells can be achieved (Di Meo C. et al., 2007, 2008; Platt V. M. et al., 2008) (Figure 2.11).

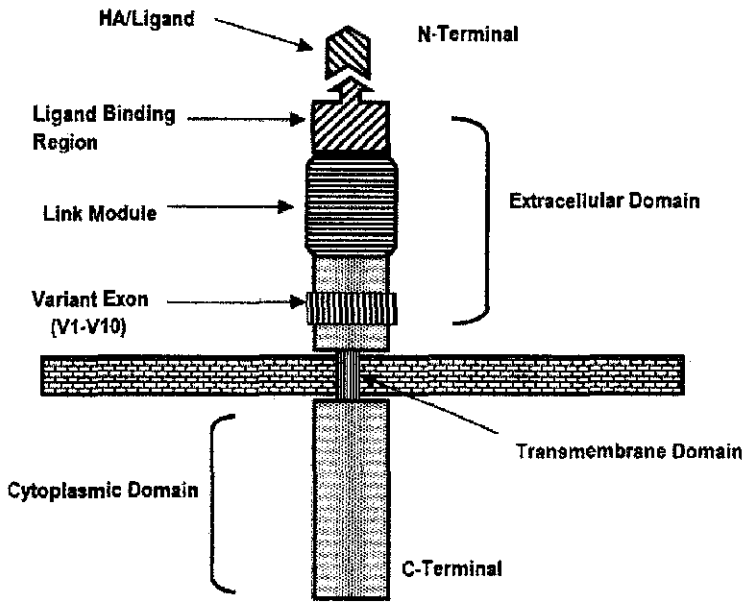


Figure 2.13 Schematic representation of CD44 molecule (Gul-e-Saba et al., 2010)

2.5 POLY (ETHYLENE GLYCOL) – PEG

PEG is the common abbreviation for polyethylene glycol – or, more properly, poly (ethylene glycol) which refers to a chemical compound composed of repeating ethylene glycol units

Poly(ethylene glycol) has several chemical properties that make it especially useful in various biological, chemical and pharmaceutical settings.

- Non-toxic and non-immunogenic – can be added to media and attached to surfaces and conjugated to molecules without interfering with cellular functions or target immunogenicities
- Hydrophilic (aqueous-soluble) – attachment to proteins and other biomolecules decreases aggregation and increases solubility
- Highly flexible – provides for surface treatment or bioconjugation without steric hindrance (www.piercenet.com, 2011).

CHAPTER 3

METHODOLOGY

3.1 RESEARCH METHODOLOGY

The study has been divided into 2 phases for FYP I and FYP II as follows:

3.1.1 FYP I PROJECT ACTIVITIES

The uni-molecular modeling had been modeled separately using Discovery Studios 2.5 (Accelrys Inc., USA).

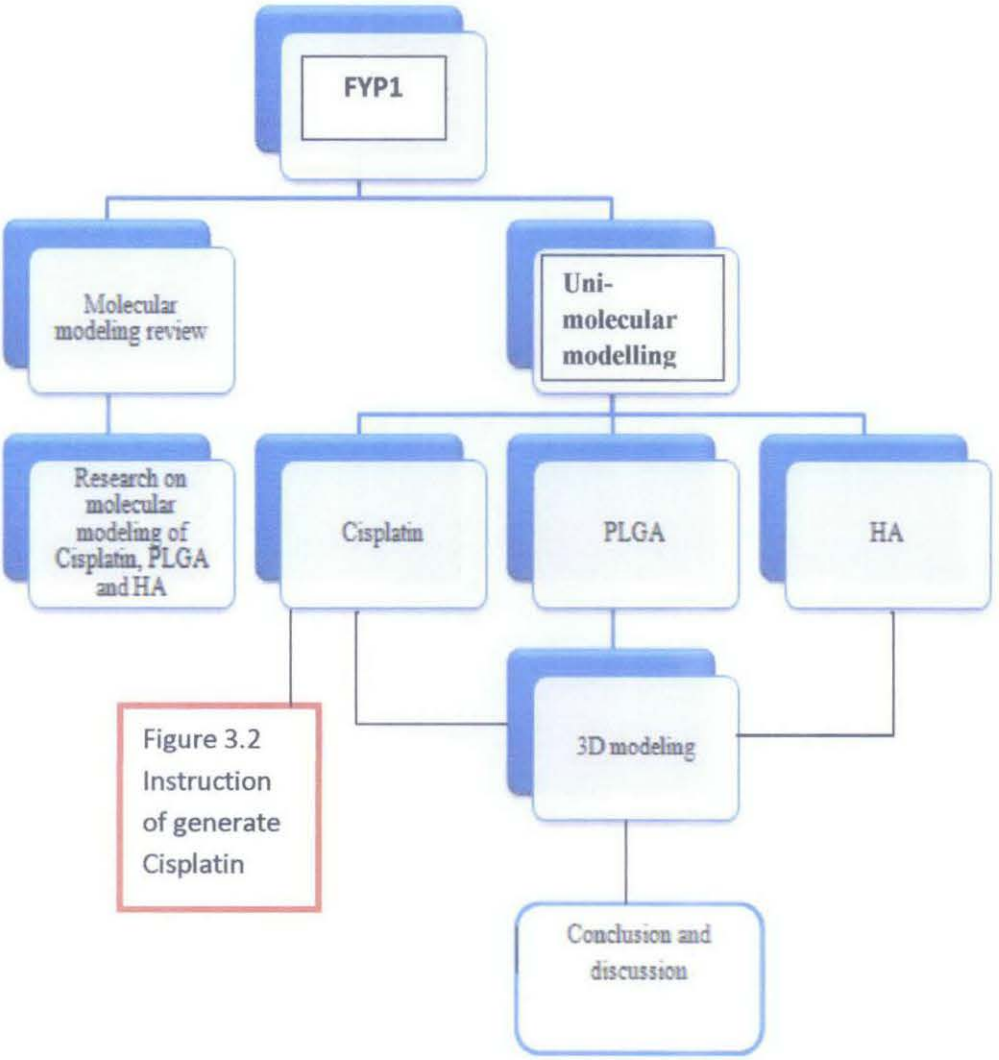
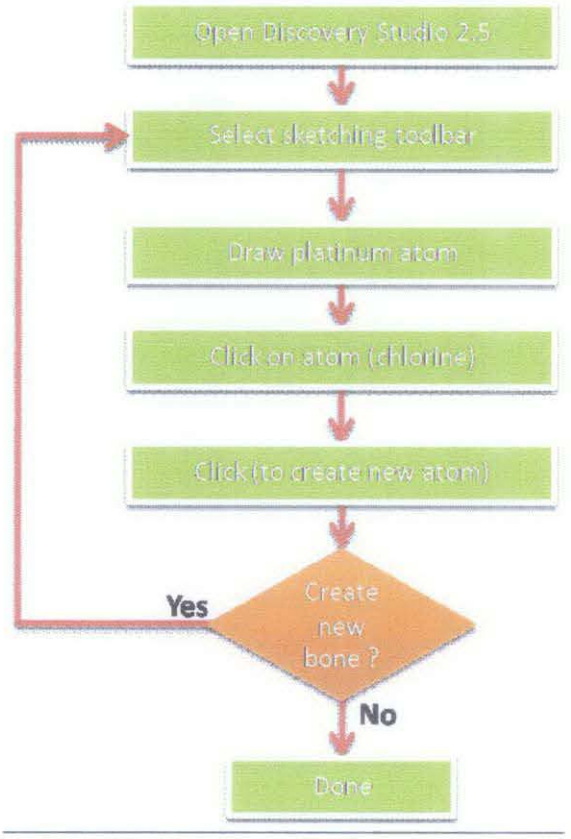


Figure 3.2 Instruction of generate Cisplatin

Figure 3.1 FYP 1 Project activities (Research on molecular modeling of cisplatin PLGA and HA and the uni-molecular modeling had been modeled)

The 3D modeling of anticancer agent (Cisplatin)



The study chose Cisplatin to be modeled (refer to Fig.2.4) and the following shows its modeling instruction:

1) Discovery Studios 2.5 was opened by using its software icon on the desktop.
2) Molecule window was opened from menu File | New | Molecule Window.
3) The Draw Tool was selected from Sketching Toolbar.
4) The Platinum atoms was drawn as the center atom, the lower left atom(Chlorine) was clicked then the mouse was dragged to lower left and single click was pressed after that to make a new atom and by clicking on the new atom, it released the cursor. Then, the upper left atom (Chlorine) was clicked and the mouse was dragged to upper left side with single click. Now the 2 Chlorine atoms were completed.

5) The upper and lower bond of Nitrogen was drawn by using the previous method. The 3 hydrogen atom was drawn by dragged 3 hydrogen from Nitrogen atom.
6) Optimizing the geometry of the structure was needed since the drawing model was not yet precise and accurate in terms of standard bond length, bond angles and Van der Waals radii for close contact. The Clean Geometry was chose from Chemistry Toolbar; this can be clicked repeatedly until there's no longer changing in the model.
7) Annotation to the model was needed by choosing Annotation Tool in Sketching Toolbar and left clicking on the place where to put the annotation then the word 'Cisplatin' was typed in the dialog box and finally the 'OK' button was pressed.

Figure 3.2 Instruction of generate a 3D uni- molecular modeling of Cisplatin The completed model of Cisplatin PLGA PEG-bis-amine (as spacer arm of PLGA conjugated HA) and HA has illustrated in the next chapter (Fig 4.1, 4.2, 4.3, and 4.4).

3.1.2 FYP II PROJECT ACTIVITIES

Multi-molecular modeling of Cisplatin-HA, PLGA-PEG-HA and Cisplatin-PLGA-PEG-HA had been modeled. Instruction of generate these conjugated molecules is by making a bond linked between those molecules. The bond length and basic properties of these molecule also been extracted as results in the next chapter.

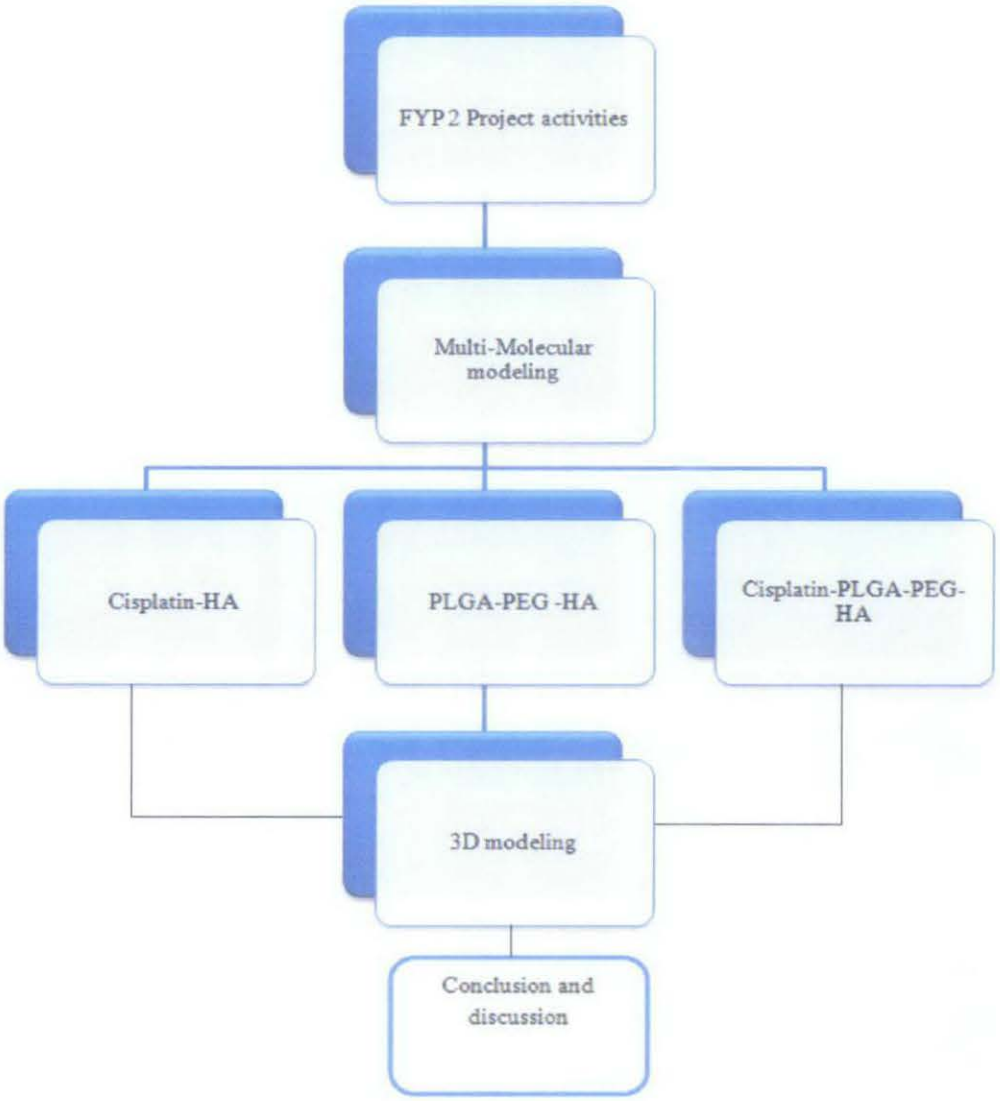


Figure 3.3 FYP 2 project activities (Multi-molecular modeling had been modeled by using Discovery Studios 2.5 (Accelrys Inc., USA))

3.2 TOOLS REQUIREMENT

Tools requirement as for development of the molecular modeling are shown below.

- Discovery Studios 2.5 (Accelrys Inc., USA)

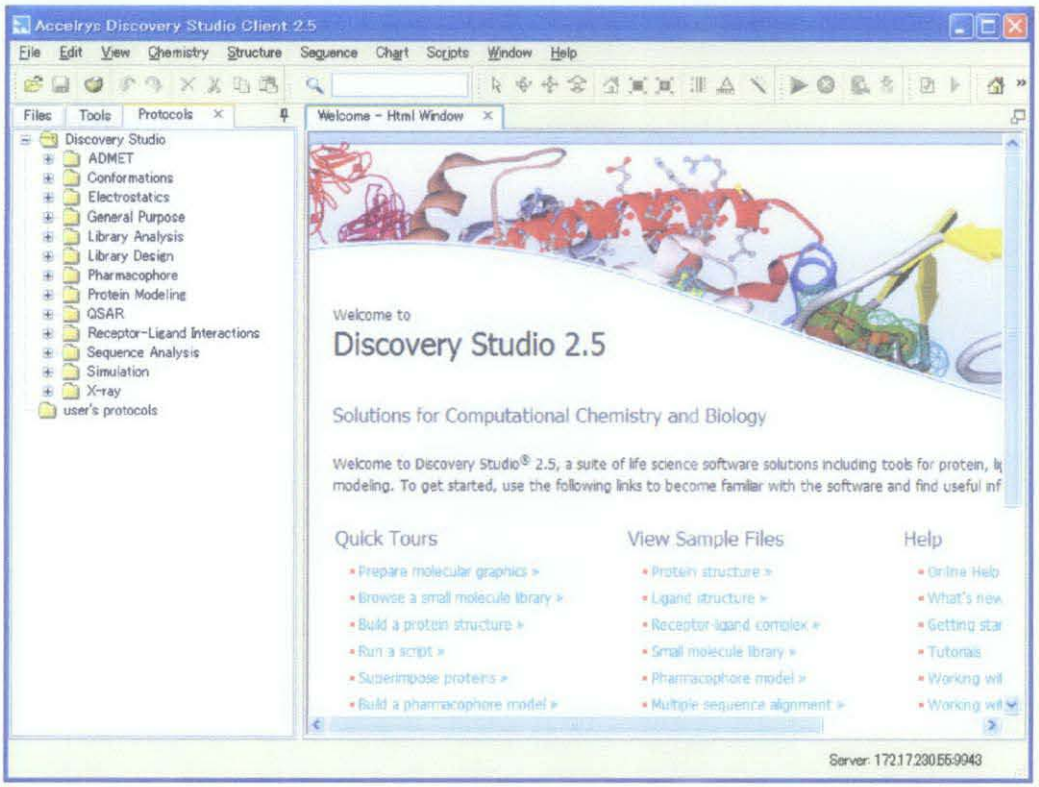


Figure 3.4 User interface of Discovery Studios 2.5 (Accelrys Inc., USA).

3.3 PROJECT TIMELINE

Activities in FYP I	W1 15/5	W2 22/5	W3 29/5	W4 5/6	W5 12/6	W6 19/6	W7 26/6	W8 3/7	W9 17/7	W 10 24/7	W 11 31/7	W 12 7/8	W 13 14/8	W 14 21/8	W 15 28/8	W 16 4/9	W 17 11/9
Selection of project topic																	
Preliminary research work																	
a) Literature review: Modeling of HA containing Cisplatin-loaded PLGA nanosphere bioconjugates for targeted delivery to cancer cells																	
Submission of extended proposal																	
Proposal defense																	
Project work continues																	
b) Execute the uni-molecular modeling																	
c) Execute the multi-molecular modeling																	
Submission of Interim Draft Report																	
Submission of Interim Report																	

Study week

Final Examination Week

Final Examination Week

Table 3.1: Final Year Project I (May 2011) proposed activities timeline.

Table 3.2: Final Year Project II (September 2011) proposed activities timeline.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 MOLECULAR MODELING

The following content illustrate the molecular modeling developed by using Discovery Studios 2.5 (Accelrys Inc., USA).

4.1.1 Uni-molecular Modeling

Fig. 4.1, 4.2, 4.3 and 4.4 show uni-molecular structure of cisplatin, a carrier molecule (PLGA), Poly(ethylene glycol) bis(amine) – PEG and Hyaluronic acid (HA) respectively. Detail bond length amongst the atoms of these uni-molecular modeling are shown in Table 4.1, 4.2, 4.3 and 4.4 respectively.

(1) An anticancer agent (Cisplatin)

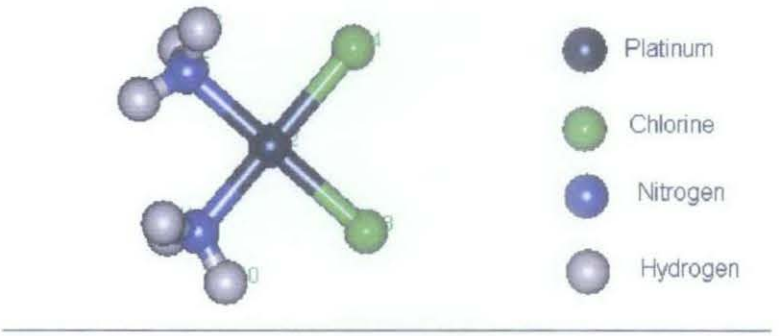


Figure 4.1 Molecular modeling of Cisplatin with each atom numbered.

Table 4.1: Basic properties of cisplatin

Name	No. of atoms	Molecular formula	Molecular composition	Molecular weight	Exact molecular weight	Net formal charge
Cisplatin	11	Cl2 H6 N2 Pt	Cl: 0.236, H: 0.020, N: 0.093, Pt: 0.650	300.058	298.956	0

Table 4.2: Bond and length of cisplatin

No	Name	Parent	Order	Type	Length
1	N1 - Pt2	Cisplatin	1	Single	2.00447
2	Pt2 - Cl3	Cisplatin	1	Single	2.30153
3	Pt2 - Cl4	Cisplatin	1	Single	2.30062
4	Pt2 - N5	Cisplatin	1	Single	2.00341
5	N5 - H6	Cisplatin	1	Single	1.02963
6	N5 - H7	Cisplatin	1	Single	1.02998
7	N5 - H8	Cisplatin	1	Single	1.02946
8	N1 - H9	Cisplatin	1	Single	1.02944
9	N1 - H10	Cisplatin	1	Single	1.02941
10	N1 - H11	Cisplatin	1	Single	1.0297

Figure 4.1 shows modeling of Cisplatin with each atom numbered. Cisplatin structure shows the total measurements of bond length =11 with average bond length = 1.478765.The table 4.1 shows modeling of Cisplatin the total number of atom is 11 with molecular composition Cl: 0.236, H: 0.020, N: 0.093, Pt: 0.650 and net formal charge =0.

(2) A carrier molecule (PLGA)

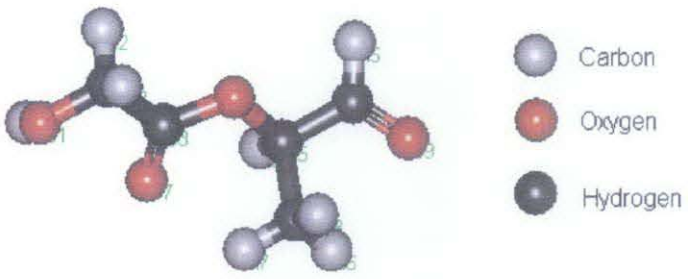


Figure 4.2 Molecular modeling of PLGA

Table 4.3: Basic properties of PLGA

Name	No. of atoms	Molecular formula	Molecular composition	Molecular weight	Exact molecular weight	Net formal charge
PLGA	18	C5 H8 O5	C: 0.405, H: 0.054, O: 0.540	148.119	148.037	0

Table 4.4: Bond and length of PLGA

No	Name	Parent	Order	Type	Length
1	O1 - C2	PLGA	1	Single	1.42944
2	C2 - C3	PLGA	1	Single	1.50007
3	C3 - O4	PLGA	1	Single	1.40999
4	O4 - C5	PLGA	1	Single	1.44509
5	C5 - C6	PLGA	1	Single	1.5004
6	C3 - O7	PLGA	2	Double	1.23722
7	C5 - C8	PLGA	1	Single	1.55092
8	C6 - O9	PLGA	2	Double	1.23615
9	O1 - H1	PLGA	1	Single	1.05003
10	C2 - H2	PLGA	1	Single	1.09
11	C2 - H3	PLGA	1	Single	1.09
12	C5 - H4	PLGA	1	Single	1.09
13	C6 - H5	PLGA	1	Single	1.09
14	C8 - H6	PLGA	1	Single	1.08996
15	C8 - H7	PLGA	1	Single	1.09003
16	C8 - H8	PLGA	1	Single	1.09003

Figure 4.2 shows modeling of PLGA with each atom numbered. PLGA structure shows the total measurements of bond length= 16 with average bond length = 1.249333.The table 4.3 shows modeling of PLGA, the total number of atom is 18 with molecular composition C: 0.405, H: 0.054, O: 0.540 and net formal charge =0.

(3) Poly(ethylene glycol) bis(amine) – PEG

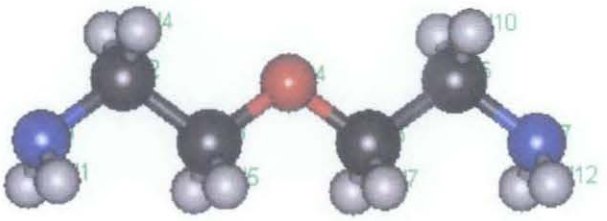


Figure 4.3 Molecular modeling of Poly(ethylene glycol) bis(amine) – PEG

Table 4.5: Basic properties of Poly(ethylene glycol) bis(amine)

Name	No. of atoms	Molecular formula	Molecular composition	Molecular weight	Exact molecular weight	Net formal charge
Poly(ethylene glycol) bis(amine)	19	C4 H12 N2 O	C: 0.461, H: 0.116, N: 0.269, O: 0.154	104.154	104.095	0

Table 4.6: Bond and length of Poly(ethylene glycol) bis(amine)-PEG

No	Name	Parent	Order	Type	Length
1	N1 - C2	Poly(ethylene glycol) bis(amine)	1	Single	1.46616
2	C2 - C3	Poly(ethylene glycol) bis(amine)	1	Single	1.53352
3	C3 - O4	Poly(ethylene glycol) bis(amine)	1	Single	1.42302
4	O4 - C5	Poly(ethylene glycol) bis(amine)	1	Single	1.42302
5	C5 - C6	Poly(ethylene glycol) bis(amine)	1	Single	1.53352
6	C6 - N7	Poly(ethylene glycol) bis(amine)	1	Single	1.46616
7	N1 - H1	Poly(ethylene glycol) bis(amine)	1	Single	1.02949
8	N1 - H2	Poly(ethylene glycol) bis(amine)	1	Single	1.02949
9	C2 - H3	Poly(ethylene glycol) bis(amine)	1	Single	1.09939
10	C2 - H4	Poly(ethylene glycol) bis(amine)	1	Single	1.09939
11	C3 - H5	Poly(ethylene glycol) bis(amine)	1	Single	1.09913
12	C3 - H6	Poly(ethylene glycol) bis(amine)	1	Single	1.09913
13	C5 - H7	Poly(ethylene glycol) bis(amine)	1	Single	1.09913
14	C5 - H8	Poly(ethylene glycol) bis(amine)	1	Single	1.09913
15	C6 - H9	Poly(ethylene glycol) bis(amine)	1	Single	1.09939
16	C6 - H10	Poly(ethylene glycol) bis(amine)	1	Single	1.09939
17	N7 - H11	Poly(ethylene glycol) bis(amine)	1	Single	1.02949
18	N7 - H12	Poly(ethylene glycol) bis(amine)	1	Single	1.02949

Figure 4.3 shows modeling of Poly(ethylene glycol) bis(amine) – PEG with each atom numbered. PLGA structure shows the total measurements of bond length = 18 with average bond length = 1.208747.The table 4.5 shows modeling of PLGA, the total number of atom is 18 with molecular composition C: 0.461, H: 0.116, N: 0.269, O: 0.154and net formal charge =0.

(4) Hyaluronic acid (HA)

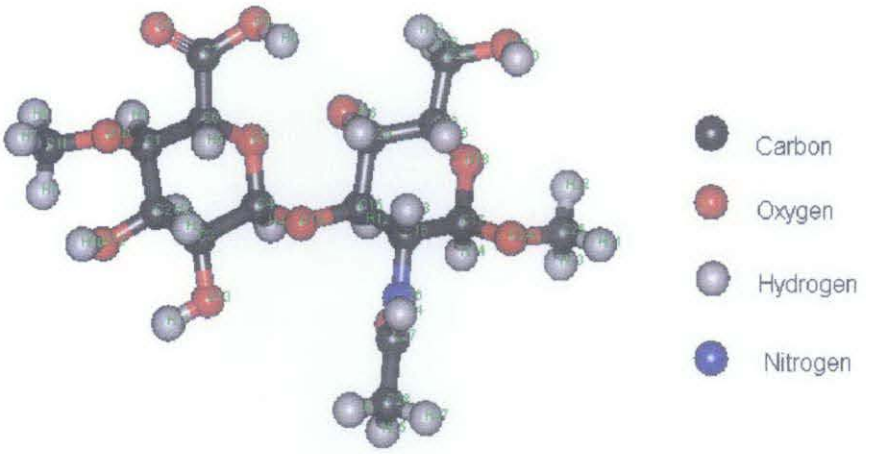


Figure 4.4 Hyaluronic acid (HA)

Table 4.7: Basic properties of Hyaluronic acid

Name	No. of atoms	Molecular formula	Molecular composition	Molecular weight	Exact molecular weight	Net formal charge
Hyaluronic Acid	56	C16 H27 N O12	C: 0.452, H: 0.064, N: 0.033, O: 0.451	425.399	425.153	0

Table 4.8: Bond and length of Hyaluronic acid(HA)

No	Name	Parent	Order	Type	Length
1	C1 - C2	Hyaluronic Acid	1	Single	1.59635
2	C2 - C3	Hyaluronic Acid	1	Single	1.4931
3	C3 - C4	Hyaluronic Acid	1	Single	1.59474
4	C4 - O5	Hyaluronic Acid	1	Single	1.48098
5	O5 - C6	Hyaluronic Acid	1	Single	1.38242
6	C6 - C1	Hyaluronic Acid	1	Single	1.55595
7	C6 - C7	Hyaluronic Acid	1	Single	1.50029
8	C7 - O8	Hyaluronic Acid	2	Double	1.23601

No	Name	Parent	Order	Type	Length
9	C7 - O9	Hyaluronic Acid	1	Single	1.39077
10	C1 - O10	Hyaluronic Acid	1	Single	1.4566
11	O10 - C11	Hyaluronic Acid	1	Single	1.43783
12	C2 - O12	Hyaluronic Acid	1	Single	1.42998
13	C3 - O13	Hyaluronic Acid	1	Single	1.42868
14	C4 - O14	Hyaluronic Acid	1	Single	1.45673
15	O14 - C15	Hyaluronic Acid	1	Single	1.45659
16	C15 - C16	Hyaluronic Acid	1	Single	1.49247
17	C16 - C17	Hyaluronic Acid	1	Single	1.60289
18	C17 - O18	Hyaluronic Acid	1	Single	1.4804
19	O18 - C19	Hyaluronic Acid	1	Single	1.37854
20	C19 - C20	Hyaluronic Acid	1	Single	1.56016
21	C20 - C15	Hyaluronic Acid	1	Single	1.57294
22	C20 - O21	Hyaluronic Acid	1	Single	1.42935
23	C19 - C22	Hyaluronic Acid	1	Single	1.53752
24	C22 - O23	Hyaluronic Acid	1	Single	1.42903
25	C17 - O24	Hyaluronic Acid	1	Single	1.43032
26	O24 - C25	Hyaluronic Acid	1	Single	1.43136
27	C16 - N26	Hyaluronic Acid	1	Single	1.45817
28	N26 - C27	Hyaluronic Acid	1	Single	1.34361
29	C27 - C28	Hyaluronic Acid	1	Single	1.49999
30	C27 - O29	Hyaluronic Acid	2	Double	1.23771
31	C1 - H1	Hyaluronic Acid	1	Single	1.09
32	C2 - H2	Hyaluronic Acid	1	Single	1.09
33	C3 - H3	Hyaluronic Acid	1	Single	1.09
34	C4 - H4	Hyaluronic Acid	1	Single	1.09
35	C6 - H5	Hyaluronic Acid	1	Single	1.09
36	O9 - H6	Hyaluronic Acid	1	Single	1.05003
37	C11 - H7	Hyaluronic Acid	1	Single	1.08996
38	C11 - H8	Hyaluronic Acid	1	Single	1.09003
39	C11 - H9	Hyaluronic Acid	1	Single	1.09003
40	O12 - H10	Hyaluronic Acid	1	Single	1.05003
41	O13 - H11	Hyaluronic Acid	1	Single	1.05003
42	C15 - H12	Hyaluronic Acid	1	Single	1.09
43	C16 - H13	Hyaluronic Acid	1	Single	1.09
44	C17 - H14	Hyaluronic Acid	1	Single	1.09
45	C19 - H15	Hyaluronic Acid	1	Single	1.09
46	C20 - H16	Hyaluronic Acid	1	Single	1.09
47	O21 - H17	Hyaluronic Acid	1	Single	1.05003
48	C22 - H18	Hyaluronic Acid	1	Single	1.09
49	C22 - H19	Hyaluronic Acid	1	Single	1.09
50	O23 - H20	Hyaluronic Acid	1	Single	1.05003
51	C25 - H21	Hyaluronic Acid	1	Single	1.08996
52	C25 - H22	Hyaluronic Acid	1	Single	1.09003

No	Name	Parent	Order	Type	Length
53	C25 - H23	Hyaluronic Acid	1	Single	1.09003
54	N26 - H24	Hyaluronic Acid	1	Single	1.07
55	C28 - H25	Hyaluronic Acid	1	Single	1.08996
56	C28 - H26	Hyaluronic Acid	1	Single	1.09003
57	C28 - H27	Hyaluronic Acid	1	Single	1.09003

Figure 4.4 shows modeling of Hyaluronic Acid with each atom numbered. Hyaluronic Acid structure shows the total measurements of bond length = 57 with average bond length = 1.280556.The table 4.7 shows modeling of Hyaluronic Acid, the total number of atom is 56 with molecular composition C: 0.452, H: 0.064, N: 0.033, O: 0.451 and net formal charge =0.

4.1.2 Multi-molecular Modeling

(1) Cisplatin- Hyaluronic Acid(HA)

Figure 4.5 Molecular modeling of Cisplatin- Hyaluronic Acid (HA)

Table 4.9: Bond and length of Cisplatin- Hyaluronic Acid (HA)

No	Name	Parent	Order	Type	Length
1	C1 - C2	Hyaluronic Acid	1	Single	1.6502
2	C2 - C3	Hyaluronic Acid	1	Single	1.43317
3	C3 - C4	Hyaluronic Acid	1	Single	1.64526
4	C4 - O5	Hyaluronic Acid	1	Single	1.52019
5	O5 - C6	Hyaluronic Acid	1	Single	1.31957
6	C6 - C1	Hyaluronic Acid	1	Single	1.587
7	C6 - C7	Hyaluronic Acid	1	Single	1.48824
8	C7 - O8	Hyaluronic Acid	2	Double	1.23553
9	C7 - O9	Hyaluronic Acid-Cisplatin	1	Single	1.38546
10	C1 - O10	Hyaluronic Acid	1	Single	1.45754
11	O10 - C11	Hyaluronic Acid	1	Single	1.44214
12	C2 - O12	Hyaluronic Acid	1	Single	1.42787
13	C3 - O13	Hyaluronic Acid	1	Single	1.42555
14	C4 - O14	Hyaluronic Acid	1	Single	1.45316
15	O14 - C15	Hyaluronic Acid	1	Single	1.45878

No	Name	Parent	Order	Type	Length
16	C15 - C16	Hyaluronic Acid	1	Single	1.45045
17	C16 - C17	Hyaluronic Acid	1	Single	1.66735
18	C17 - O18	Hyaluronic Acid	1	Single	1.522
19	O18 - C19	Hyaluronic Acid	1	Single	1.31852
20	C19 - C20	Hyaluronic Acid	1	Single	1.57794
21	C20 - C15	Hyaluronic Acid	1	Single	1.62192
22	C20 - O21	Hyaluronic Acid	1	Single	1.43608
23	C19 - C22	Hyaluronic Acid	1	Single	1.5295
24	C22 - O23	Hyaluronic Acid	1	Single	1.4268
25	C17 - O24	Hyaluronic Acid	1	Single	1.42985
26	O24 - C25	Hyaluronic Acid	1	Single	1.42981
27	C16 - N26	Hyaluronic Acid	1	Single	1.46658
28	N26 - C27	Hyaluronic Acid	1	Single	1.34449
29	C27 - C28	Hyaluronic Acid	1	Single	1.49545
30	C27 - O29	Hyaluronic Acid	2	Double	1.23692
31	C1 - H1	Hyaluronic Acid	1	Single	1.09844
32	C2 - H2	Hyaluronic Acid	1	Single	1.09904
33	C3 - H3	Hyaluronic Acid	1	Single	1.10309
34	C4 - H4	Hyaluronic Acid	1	Single	1.09827
35	C6 - H5	Hyaluronic Acid	1	Single	1.10369
36	C11 - H7	Hyaluronic Acid	1	Single	1.09886
37	C11 - H8	Hyaluronic Acid	1	Single	1.08686
38	C11 - H9	Hyaluronic Acid	1	Single	1.09939
39	O12 - H10	Hyaluronic Acid	1	Single	0.979642
40	O13 - H11	Hyaluronic Acid	1	Single	0.988695
41	C15 - H12	Hyaluronic Acid	1	Single	1.09635
42	C16 - H13	Hyaluronic Acid	1	Single	1.10112
43	C17 - H14	Hyaluronic Acid	1	Single	1.0964
44	C19 - H15	Hyaluronic Acid	1	Single	1.10274
45	C20 - H16	Hyaluronic Acid	1	Single	1.09685
46	O21 - H17	Hyaluronic Acid	1	Single	0.986659
47	C22 - H18	Hyaluronic Acid	1	Single	1.09906
48	C22 - H19	Hyaluronic Acid	1	Single	1.09898
49	O23 - H20	Hyaluronic Acid	1	Single	0.989383
50	C25 - H21	Hyaluronic Acid	1	Single	1.09905
51	C25 - H22	Hyaluronic Acid	1	Single	1.0995
52	C25 - H23	Hyaluronic Acid	1	Single	1.09936
53	N26 - H24	Hyaluronic Acid	1	Single	0.993188
54	C28 - H25	Hyaluronic Acid	1	Single	1.0994
55	C28 - H26	Hyaluronic Acid	1	Single	1.09956

No	Name	Parent	Order	Type	Length
56	C28 - H27	Hyaluronic Acid	1	Single	1.09944
57	N1 - Pt2	Cisplatin	1	Single	2.00346
58	Pt2 - Cl4	Cisplatin	1	Single	2.30115
59	Pt2 - N5	Cisplatin	1	Single	2.00314
60	N5 - H6	Cisplatin	1	Single	1.0295
61	N5 - H7	Cisplatin	1	Single	1.03012
62	N5 - H8	Cisplatin	1	Single	1.02932
63	N1 - H9	Cisplatin	1	Single	1.03098
64	N1 - H10	Cisplatin	1	Single	1.02911
65	N1 - H11	Cisplatin	1	Single	1.02942
66	Pt2 - O9	Cisplatin	1	Single	1.95687

Figure 4.5 shows modeling of Cisplatin- Hyaluronic Acid (HA) with each atom numbered. Cisplatin- Hyaluronic Acid (HA) structure shows the total measurements of bond length = 66 with average bond length = 1.308173. HA is found naturally inside human body, highly viscous and a versatile polymer. Due to its biocompatibility and hydrophilic nature, the problem of poor solubility of naturally occurring, cytotoxic and hydrophobic anticancer agents can be overcome which open up avenues for anticancer agent delivery A glycoprotein CD44 receptor, normally overexpressed in cancer cells, can serve as a major receptor protein for HA (Gul-e-Saba et al., 2011). Cisplatin, classified as an alkylating agent, is the first platinum-based anticancer drug. It inhibits synthesis or multiplication of host DNA by formation of DNA adducts that result in cell death (Gul-e-Saba et al., 2011). Inside the cells, HA undergoes degradation and drug will be released (Gul-e-Saba et al., 2011). Released cisplatin undergoes hydrolysis, producing the highly reactive charged platinum complex $[Pt\ (NH_3)_2ClH_2O]^+$ in the first step hydrolysis with the substitution of one Cl-atom with water molecule. Second step hydrolysis produced highly oxidized form of cisplatin by substitution of second Cl-atom with water molecule. This Pt complex interacts with DNA through the N7 atom of either a guanine or adenine base. Further hydrolysis displaces the remaining chloride ligand, and the platinum can bind to a second nucleotide base. The cisplatin-DNA adduct is recognized by a high mobility group (HMG)-domain protein, among other DNA repair proteins, which binds tightly to the complex. The adduct causes destacking of the nucleotide bases, resulting in the DNA helix becoming kinked (Trzaska, 2005).

(2) PLGA-PEG-bis-amine- Hyaluronic Acid(HA)

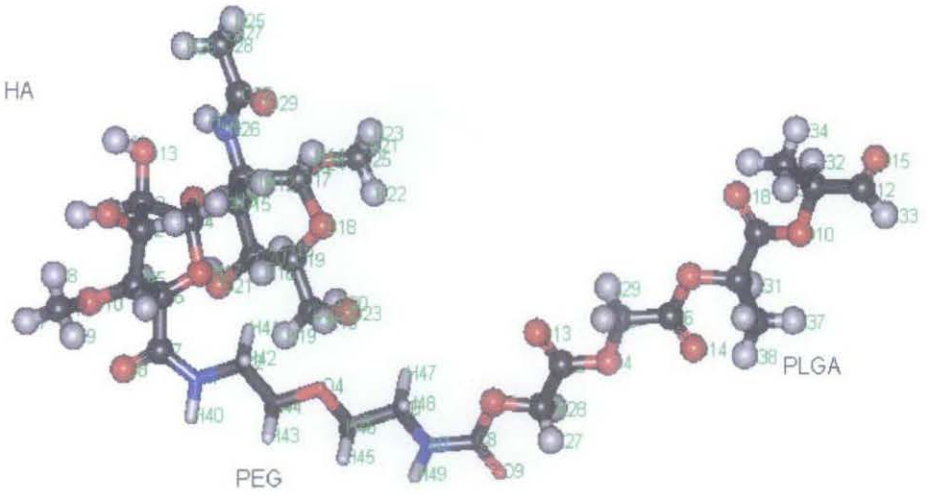


Figure 4.6 Molecular modeling of PLGA-PEG-bis-amine- Hyaluronic Acid (HA)

Table 4.10: Bond and length of PLGA-PEG-bis-amine- Hyaluronic Acid (HA)

No.	Name	Parent	Order	Type	Length (Å)
1	C1 - C2	Hyaluronic Acid	1	Single	1.65421
2	C2 - C3	Hyaluronic Acid	1	Single	1.43798
3	C3 - C4	Hyaluronic Acid	1	Single	1.64219
4	C4 - O5	Hyaluronic Acid	1	Single	1.51794
5	O5 - C6	Hyaluronic Acid	1	Single	1.32966
6	C6 - C1	Hyaluronic Acid	1	Single	1.58803
7	C6 - C7	Hyaluronic Acid	1	Single	1.54045
8	C7 - O8	Hyaluronic Acid	2	Double	1.23577
9	C1 - O10	Hyaluronic Acid	1	Single	1.45877
10	O10 - C11	Hyaluronic Acid	1	Single	1.44341
11	C2 - O12	Hyaluronic Acid	1	Single	1.4279
12	C3 - O13	Hyaluronic Acid	1	Single	1.42562
13	C4 - O14	Hyaluronic Acid	1	Single	1.45506
14	O14 - C15	Hyaluronic Acid	1	Single	1.46159
15	C15 - C16	Hyaluronic Acid	1	Single	1.45083
16	C16 - C17	Hyaluronic Acid	1	Single	1.66793
17	C17 - O18	Hyaluronic Acid	1	Single	1.52218

No.	Name	Parent	Order	Type	Length (Å)
18	O18 - C19	Hyaluronic Acid	1	Single	1.31802
19	C19 - C20	Hyaluronic Acid	1	Single	1.57516
20	C20 - C15	Hyaluronic Acid	1	Single	1.61966
21	C20 - O21	Hyaluronic Acid	1	Single	1.43594
22	C19 - C22	Hyaluronic Acid	1	Single	1.53067
23	C22 - O23	Hyaluronic Acid	1	Single	1.42623
24	C17 - O24	Hyaluronic Acid	1	Single	1.42983
25	O24 - C25	Hyaluronic Acid	1	Single	1.42979
26	C16 - N26	Hyaluronic Acid	1	Single	1.46651
27	N26 - C27	Hyaluronic Acid	1	Single	1.34448
28	C27 - C28	Hyaluronic Acid	1	Single	1.49547
29	C27 - O29	Hyaluronic Acid	2	Double	1.2369
30	C1 - H1	Hyaluronic Acid	1	Single	1.0983
31	C2 - H2	Hyaluronic Acid	1	Single	1.09903
32	C3 - H3	Hyaluronic Acid	1	Single	1.10268
33	C4 - H4	Hyaluronic Acid	1	Single	1.09819
34	C6 - H5	Hyaluronic Acid	1	Single	1.10187
35	C11 - H7	Hyaluronic Acid	1	Single	1.09891
36	C11 - H8	Hyaluronic Acid	1	Single	1.08611
37	C11 - H9	Hyaluronic Acid	1	Single	1.099
38	O12 - H10	Hyaluronic Acid	1	Single	0.979109
39	O13 - H11	Hyaluronic Acid	1	Single	0.988776
40	C15 - H12	Hyaluronic Acid	1	Single	1.09638
41	C16 - H13	Hyaluronic Acid	1	Single	1.10112
42	C17 - H14	Hyaluronic Acid	1	Single	1.09635
43	C19 - H15	Hyaluronic Acid	1	Single	1.10271
44	C20 - H16	Hyaluronic Acid	1	Single	1.09509
45	O21 - H17	Hyaluronic Acid	1	Single	0.986539
46	C22 - H18	Hyaluronic Acid	1	Single	1.10032
47	C22 - H19	Hyaluronic Acid	1	Single	1.09901
48	O23 - H20	Hyaluronic Acid	1	Single	0.989312
49	C25 - H21	Hyaluronic Acid	1	Single	1.09903
50	C25 - H22	Hyaluronic Acid	1	Single	1.09954
51	C25 - H23	Hyaluronic Acid	1	Single	1.0993
52	N26 - H24	Hyaluronic Acid	1	Single	0.993187
53	C28 - H25	Hyaluronic Acid	1	Single	1.09942
54	C28 - H26	Hyaluronic Acid	1	Single	1.09951
55	C28 - H27	Hyaluronic Acid	1	Single	1.09947
56	N1 - C7	PEG-HA	1	Single	1.34936
57	N1 - C2	PEG-bis amine	1	Single	1.43977
58	C2 - C3	PEG-bis amine	1	Single	1.53534
59	C3 - O4	PEG-bis amine	1	Single	1.42819
60	O4 - C5	PEG-bis amine	1	Single	1.42779
61	C5 - C6	PEG-bis amine	1	Single	1.53802

No.	Name	Parent	Order	Type	Length (Å)
62	C6 - N7	PEG-bis amine	1	Single	1.43419
63	N7 - C8	PEG-bis amine	1	Single	1.39519
64	C8 - O9	PEG-bis amine	2	Double	1.23641
65	N1 - H40	PEG-bis amine	1	Single	1.07
66	C2 - H41	PEG-bis amine	1	Single	1.09
67	C2 - H42	PEG-bis amine	1	Single	1.09
68	C3 - H43	PEG-bis amine	1	Single	1.09
69	C3 - H44	PEG-bis amine	1	Single	1.09
70	C5 - H45	PEG-bis amine	1	Single	1.09
71	C5 - H46	PEG-bis amine	1	Single	1.09
72	C6 - H47	PEG-bis amine	1	Single	1.09
73	C6 - H48	PEG-bis amine	1	Single	1.09
74	N7 - H49	PEG-bis amine	1	Single	1.07
75	C8 - O1	PLGA-PEG	1	Single	1.39224
76	O1 - C2	PLGA	1	Single	1.43024
77	C2 - C3	PLGA	1	Single	1.49798
78	C3 - O4	PLGA	1	Single	1.39185
79	O4 - C5	PLGA	1	Single	1.42868
80	C5 - C6	PLGA	1	Single	1.49864
81	C6 - O7	PLGA	1	Single	1.4095
82	O7 - C8	PLGA	1	Single	1.44536
83	C8 - C9	PLGA	1	Single	1.49838
84	C9 - O10	PLGA	1	Single	1.40994
85	O10 - C11	PLGA	1	Single	1.44508
86	C11 - C12	PLGA	1	Single	1.50032
87	C3 - O13	PLGA	2	Double	1.23636
88	C6 - O14	PLGA	2	Double	1.23702
89	C12 - O15	PLGA	2	Double	1.23615
90	C11 - C16	PLGA	1	Single	1.55065
91	C8 - C17	PLGA	1	Single	1.55089
92	C9 - O18	PLGA	2	Double	1.23706
93	C2 - H27	PLGA	1	Single	1.09
94	C2 - H28	PLGA	1	Single	1.09
95	C5 - H29	PLGA	1	Single	1.09
96	C5 - H30	PLGA	1	Single	1.09
97	C8 - H31	PLGA	1	Single	1.09
98	C11 - H32	PLGA	1	Single	1.09
99	C12 - H33	PLGA	1	Single	1.09
100	C16 - H34	PLGA	1	Single	1.08996
101	C16 - H35	PLGA	1	Single	1.09003
102	C16 - H36	PLGA	1	Single	1.09003
103	C17 - H37	PLGA	1	Single	1.08995
104	C17 - H38	PLGA	1	Single	1.09003
105	C17 - H39	PLGA	1	Single	1.09003

Figure 4.6 shows modeling of PLGA-PEG-bis-amine- Hyaluronic Acid (HA) with each atom numbered. PLGA-PEG-bis-amine- Hyaluronic Acid (HA) structure shows the total measurements of bond length = 105 with average bond length = 1.274163. The PLGA polymer had several advantages like good mechanical properties, low immunogenicity and toxicity, excellent biocompatibility and predictable biodegradation kinetics. The wide acceptance of the lactide/glycolide polymers as suture materials made them attractive candidates for biomedical applications like ligament reconstruction, tracheal replacement, surgical dressings, vascular grafts and nerve, dental and fracture repairs (Lewis et al.,1990; Visscher et al., 1988).The PEG-bis-amine act as the spacer arm to connect the PLGA together with HA. HA-conjugates often target CD44 glycoprotein, a receptor on cancer cells. CD44 has been suggested as one of the important surface markers for both normal stem cells and cancer stem cells (Al-Haj et al., 2003). HA interactions with CD44 mediate at least three important physiological processes - signal transduction, assembly of pericellular matrices, and receptor-mediated internalization (Toole, 2001; Knudson et al., 2002) Nanoparticulate delivery systems strategy may allow a controlled release of the drug and a high targeting selectivity on tumor cells, increasing drug cytotoxicity and decreasing its undesirable side effects (Serafino et al., 2011).

(3) Cisplatin-PLGA-PEG-bis-amine- Hyaluronic Acid(HA)

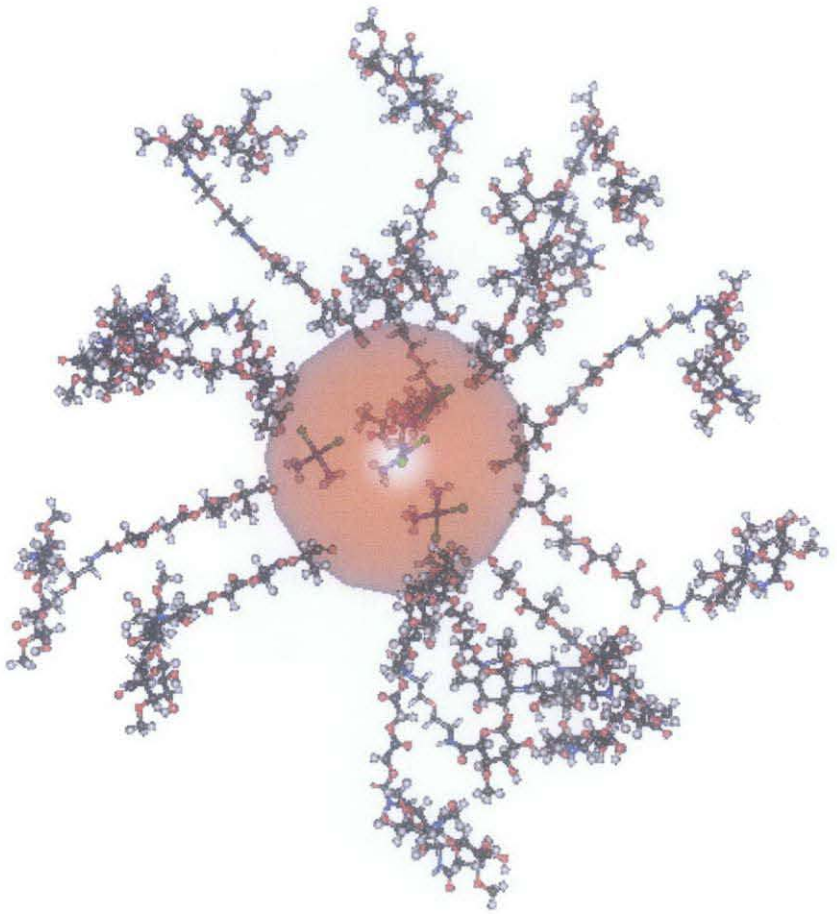


Figure 4.7 Spherical structure of PLGA-PEG-HA surrounding Cisplatin molecule.

Poor water solubility has always been one of the most fundamental problems in drug delivery (Patel, 2011). It is estimated that around 40% of drugs in the pipeline cannot be delivered through the preferred route or in some cases, at all, owing to poor water solubility (Salata, 2004). Bioconjugates of low molecular weight HA with cytotoxic agents is designed to improve solubility of the cytotoxic agent and facilitate its intravenous administration (Brekke & Gubbe, 2008). The mechanical strength, swelling behavior, capacity to undergo hydrolysis and, subsequently, the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The resultant crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGA polymers

containing a 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing a higher proportion of either of the two monomers (Gilding & Reed, 1979; Li & McCarthy, 1999).PGA is highly crystalline because it lacks the methyl side groups of the PLA. Lactic acid is more hydrophobic than glycolic acid and, therefore, lactide-rich PLGA co-polymers are less hydrophilic, absorb less water and, subsequently, degrade more slowly (Schliecker et al., 2003; Makino et al., 1985). Cisplatin as a free drug is sparingly soluble in water, conjugation to PLGA-PEG HA not only further increases its solubility, but more importantly it enhances the chances to reach the cancer site. As CD44 is over expressed in cancer cells, malignant cells with high metastatic activities often exhibit enhanced binding and uptake of HA With intrinsic cell specific binding capacity and strong affinity to cell-specific surface markers such as CD44 (Maeda et al., 1992) and Nanoparticulate delivery systems strategy may allow a controlled release of the drug and a high targeting selectivity on tumor cells, increasing drug cytotoxicity and decreasing its undesirable side effects (Serafino et al., 2011). Cisplatin-PLGA-PEG-HA interaction can be further modelled for CD-44 target and this could pave the way for rational design of target-specific drug carrier (Gul-e-Saba et al., 2011).

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

- Simulation studies are provide the better understanding of polymeric conjugate structure and it role in delivery of drug to targeted side in human body. The molecular 3D structural modeling of Cisplatin, PLGA, PEG, HA and conjugated molecular 3D Structures Cisplatin conjugated HA and PLGA-PEG-HA successfully completed using Discovery Studio 2.5.

- Besides this Nano spherical structure of PLGA-PEG-HA surrounding cisplatin molecule also generated to create the concept of Nano polymeric drug delivery system (PDDS).

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