LITERATURE REVIEWS

In treating the cancer cell, the method is very important because of the side effect on normal cell. This research expects to study drug delivery treatment to cancer cell. The drug delivery consists of drug and drug carrier. The drug delivery structure has several formations such as encapsulation, hydrogel, nanoaggregation and micelle.

Drug delivery

The doxorubicin (DOX) is used widely in cancer therapy. The medication is used to treat in the human organelles such as breast, ovarian, transitional cell bladder, bronchogenic lung, thyroid, gastric, soft tissue and osteogenic sarcomas, neuroblastoma, and Wilms' tumor. The treatment must be minimized the undesirable side effects. The side effects from doxorubicin are common and include: nausea and vomiting, loss appetite, diarrhea, difficult swallowing, thinned or brittle hair, skin irritation, darkening of fingernail or toenails, and swelling (U.S. National library of medicine, 2007). The efficacy of present cancer chemotherapy is mainly limited by the toxicity associated with the anticancer drugs to normal tissues. This limitation of anticancer drug is used in chemotherapy, lack efficient selectivity towards melanoma cells. The doxorubicin molecule is a member of the anthracycline ring antibiotics. The experiments are studied in efficiency to treat at the inoculation of murine macrophage cells into Balb/c mice. Mice are randomized into group such as untreated controls, empty chitosan nanoparticles, doxorubicin in solution, dextran–doxorubicin conjugate, chitosan nanoparticle entrapped dextran-doxorubicin, and chitosan nanoparticle entrapped dextran-doxorubicin conjugate. In this study, the drug conjugate was attempted to couple the drug with dextran and capsulate in hydrogel nanoparticles. The size of these nanoparticles was 100±10 nm diameter determined by quasi-elastic light scattering. In the results, the conjugation of doxorubicin with dextran effectively reduces toxicity of the free drug and optimizes the drug efficacy by its low molecular weight. But the size of the conjugate is not necessarily large enough to exploit the enhanced permeability and retention (EPR) effect of macromolecular therapeutics (Mitra *et al.*, 2001).

The doxorubicin can be used in drug delivery by conjugation with dextran. The mice are injected intravenously with both dextran-doxorubicin conjugates and the

encapsulated conjugation in chitosan nanoparticles. This drug conjugation decreases tumor volume after 4 weekly injections. The tumor volume of the mice treated with the encapsulated conjugation is lower than the tumors treated with the conjugate alone. But the treatment with doxorubicin alone did not decrease the tumor volume, because this molecule can bind both normal and cancer cell. Then, This drug has possibility to bind with other cell (Brannon-Peppas and Blanchette. 2004).

The capsule is used to deliver drug. From capsulate study, Wang *et al*. (2006) used the capsulate chitosan, Lee *et al.* (2007) used the poly(lactide)-tocopheryl polyethylene glycol succinate copolymers, and Tardi *et al*. (2006) used irrinotecan and floxuridine. In the micelle formation drug delivery, the molecule is used to form drug such as Xiangyang *et al*. (2006) used *N*-succinyl-*N*-octyl chitosan (SOC), Yang *et al*. (2006) used isocyanate terminated linear polyethylene glycol.

Janes *et al.* (2001) modified the potential of chitosan nanoparticles carrying the anthracycline drug, doxorubicin. This drug can entrap a cationic, hydrophilic molecule into nanoparticles formed by ionic elation of the positively charged polysaccharide chitosan. The experiment investigates the possibility of forming a complex between chitosan and doxorubicin prior to the formation of the particles. In the result, the complex is observed upon formation of the nanoparticles. And the drug release *in vitro* is very slow. The chitosan nanoparticles are feasibility to use in the delivery of the doxorubicin by incorporating. The polyanion is able to encapsulate considerably high quantities of doxorubicin. In consideration, the inherent polymer-drug is charge repulsion. The doxorubicin can be formed complex to chitosan.

The drug carrier usually has some functional group to detect cancer cell target. This cancer cell target is protein molecule. Son *et al*. (2003) studies an *in vivo* tumor targeting test of glycol-chitosan nanoaggregates was carried out with fluorescein isothiocyanate-conjugated glycol-chitosan nanoaggregates and the doxorubicin conjugated glycol-chitosan (GC-DOX). To investigate its biodistribution in tumor-bearing rats, the glycol-chitosan is labeled with fluorescein isothiocyanate (FITC), which formed nanoaggregates with in aqueous media. The GC-DOX nanoaggregates are acid-sensitive molecule. The GC-DOX can form micelle-like nanoaggregates spontaneously in aqueous media. The result is that doxorubicin loaded GC-DOX nanoaggregates (DOX/GC-DOX) are injected

into the tail vein of tumor-bearing rats. And their anti-tumor effect is examined by water soluble glycol-chitosan formed nanoaggregates in aqueous media by the conjugation of doxorubicin and FITC.

Boonsongrit *et al.* (2006) studied an interaction of drug and chitosan. The drug used in the study has three models such as the insulin, diclofenac sodium, and salicylic acid. These drugs and chitosan can occur by ionic interaction. These three model drugs are considered from pI and pKa to interaction between drugs and chitosan. In the result, three model drugs with different pI or pKa are studied; insulin-pI=5.3, diclofenac sodium-pKa=4.0 and salicylic acid-pKa=2.8. An increase in binding force to chitosan is expected with a decrease in pKa.

Okabe *et al.* (2005) studied specific efflux transporters, P-glycoprotein. The study showed the conferring of drug resistance by decreasing the intracellular accumulation of anticancer drugs. The doxorubicin consisted of the tetracyclic quinoid aglycone doxorubicinone linked to the aminosugar daunosamine. The daounosamine sugar can be protonated within a physiological pH range. A carrier of doxorubicin may transport the ionized form of the drug in cancer cell because the major side effects of doxorubicin are the potentially lethal cardiac toxicity.

Cheng *et al.* (2006) studied the functionalized poly(D,L-lactide–co–glycolide)–block–poly(ethylene glycol) nanoparticles for *in vivo* targeted drug delivery. The nanoparticles size has been shown to significantly affect the biodistribution of targeted and non-targeted NPs in an organ specific manner. Herein, the nanoparticles have developed from carboxy-terminated poly(D,L-lactide–co–glycolide)–block–poly(ethylene glycol) polymer. And the effects of altering the following formulation parameters on the size of NPs are studied by: 1) polymer concentration, 2) drug loading, 3) water miscibility of solvent, and 4) the ratio of water to solvent. The study found that the volumetric size of treatment correlates linearly with polymer concentration and the nanoparticles size can be controlled together with targeted delivery. These nanoparticles may be used for favorable biodistribution and development of clinically relevant targeted therapies.

Park *et al.* (2006) studied self-assembled nanoparticles, which is formed by polymeric amphiphiles. These nanoparticles of polymeric amphiphiles are been

demonstrated to accumulate in solid tumors by the enhanced permeability and retention effect. The glycol chitosan can be modified in forming nano-sized self-aggregates by chemical conjugation of doxorubicin to the backbone of glycol chitosan. The self-aggregate biodistribution is evaluated by using tissues obtained the tumor-bearing mice. When self-aggregates loaded with doxorubicin are administered into the tumor-bearing mice via the tail vein, these self-aggregates are lower toxicity than anti-tumor activity to free doxorubicin. The promising potential of self-aggregates on the basis of glycol chitosan can carry the hydrophobic anti-tumor agent.

The chitosan is poor water solubility. (Chan et al. 2007) synthesizes the chitosan-g-poly(ethylene glycol) in drug delivery. The poly(ethylene glycol) can increase the chitosan-g-poly(ethylene glycol) solubility. The folate conjugation may improve gene transfection efficiency due to promoted uptake of folate receptor bearing tumor cells. In this study, the poly(ethylene glycol) can form with folate in folate-poly(ethylene glycol)-grafted chitosan structure for targeted plasmid DNA delivery to tumor cells. From this study, the doxorubicin can be carried by chitosan capsule. This capsule can protect and carry drug. And the functional group needs use to detect the cancer cell such as poly(ethylene glycol). The poly(ethylene glycol) is a polyether diol, which is amphiphilic. This amphiphilic molecule can be dissolved in aqueous and organic solvents.

Drug release

The chitosan is a biodegradable and biocompatible polysaccharide. This chitosan sheet can be used as drug carrier for controlled release. The chitosan sheet is stable in water and degrades with lysozyme or an acidic solution *in vitro*. From study by Saito et al. (2006), the chitosan is a slightly cationic natural polysaccharide which can react with the numerous amino groups. For the drug release, the chitosan is biodegradable and can release drug to target such as urine and liver. The biodegradation of chitosan is mediated by lysozyme. This release depends on specific cells or organs into blood. The enhancement of lysozyme is probably a biological defensive function, followed by activation of the macrophage system in animals

During the release of drug from chitosan capsule, the solution has an influence on drug delivery system. The chitosan sponge delivery is prepared by freeze-drying partially

N-acetylated chitosan gels and crosslinked by glytaraldehyde in chitosan solution. From this study, the pH of dissolution media and the drug content of the sponges affected the release of drug. The drug released at pH 1.2 is faster than at pH 7.4. The release of drug can be controlled by varying the drug content, the acetylation and cross linking. The delayed drug release came from the decreased chitosan solubility by either N-acetylation or crosslinking (Oungbho and Muller. 1997).

Chung *et al.* (2005) studied the solubility of chitosan at neutral or basic pH using the Maillard-type reaction method. This study was prepared by the the water-soluble chitosans by various chitosans and saccharides. The saccharides were maltose, fructose and glucopsamine. These molecules were used under various operating conditions. The result indicated that the solubility of modified chitosan is significantly greater than that of native chitosan. The chitosan-maltose derivative remained soluble when the pH approaches 10. The chitosan-glucosamine derivative exhibited high chelating capacity in acid-soluble chitosan.

From the previous study, the chitosan capsule can release drug in acid condition such as in the stomach. This capsule can use to protect or cover the doxorubicin for cancer cell treatment. In addition, this molecule is biodegradable and can be deteriorated by lysozyme.

Micelle delivery

Kabanov *et al.* (2005) modified pluronic, the A–B–A amphiphilic block copolymers of poly(ethylene oxide) and poly(propylene oxide). The pluronic can up-regulate the expression of selected genes in cells and alter genetic responses to antineoplastic agents in cancer. Cationic lipids and polycations were used extensively for the design of nonviral gene delivery systems. The formulation of doxorubicin with the mixture of pluronic is effective against multidrug-resistant tumors, in which doxorubicin is partitioned between the micelles and bulk aqueous phase. The multidrug-resistant cancer cell is released in the external media upon dilution in body fluids. From this research, the poly(ethylene glycol) structure likes poly(ethylene oxide) and poly(propylene oxide) copolymer. Hence, the poly(ethylene glycol) is used as a drug carrier in micelle formation.

Yao *et al.* (2007) studies the chitosan derivatives with octyl, sulfate and polyethylene glycol monomethyl ether groups. This derivative of chitosan contains hydrophobic and hydrophilic moieties. The novel N-poly(ethylene glycol)-N-octyl-O-sulfate chitosan derivatives can form micelles about 100–130 nm. This micelle has potential brain-targeting characteristics. From this study, the poly(ethylene glycol) chitosan can form micelle formation. The micelle of drug delivery is very important because the drug is hydrophobic molecule, not soluble in water. The micelle protects the drug from dissolving and sends drug to the target. Beside, the micelle protects drug to dissolve form effect of other molecules in solution.

Molecular simulations

Lam *et al.* (2004) studied the hydrophobic molecules effect on the morphology of aqueous solutions of amphiphilic block copolymer, which has potential drug delivery applications. The effect was studied both experimental and simulations. Using cryogenic TEM observations, the micelles can clearly be visualised and their core sizes can be measured. While pure polymer solutions form is spherical micelles with a narrow size distribution, addition of small amounts of hydrophobic drug molecules leads to distortions in shape, a wider size distribution, and larger average core diameter. Simulations are based on a mesoscale dynamic density functional method with Gaussian chain Hamiltonian and mean-field interactions, as implemented in the MesoDyn code. From this simulation, the hydrophobic molecules can be dissolved in the micellar solution. The effect on the morphology has been investigated in detail both by cryogenic TEM and by simulations.

Sandoval *et al.* (2005) studied chitosan compound by molecular dynamics simulations method. This chitosan compounds in this simulation were chitosan/poly(vinyl aclcohol) and Chitosan/poly(2-hydroxyethyl methacrylate). This method was able to find miscibility between these polymers and attributed to hydrogen bonding formation.

Sun *et al*. (2003) used Gaussian 98 to optimize the geometries of common chemical systems. The *ab initio* method was used to simulate. The system included diatomics, N_2, O_2, F_2 and CO, and carbon based organic systems, ethane, ethylene, acetylene, 1,3-butadiene, 1,3,5-hexatriene, benzene, biphenyl, naphtalene graphene, polyethylene and all-trans-polyacetylene. The simulation based on the generalized gradient

approximation was very good agreement on bond lengths and angles as compared with experimental value.

Cummins and Gready (2003) used the semi-empirical quantum mechanics method to describe molecular interactions adequately. This simulation studied the enzymic reaction mechanism, 20 phosphate groups in NADPH cleaved from the ribose ring. In description of intermolecular, the forces in particular had interaction between atoms. This simulation associated with strong hydrogen bonding. From the study, the semi-empirical AM1 or PM3 method was used to simulate the protein systems. The method is applied to the calculation of the reaction free energy for the enzymic reduction of DHF by NADPH cofactor bound to Escherichia coli dihydrofolate reductase. The free energy change for this reduction, calculated using the configuration space sampled in a multiple molecular dynamics simulation, is found to be an encouraging agreement with the experimental results.

Frimand and Jalkanen (2002) studied in molecular simulation of propylene oxide (in Figure 1) by semi-empiracal and *ab initio* methods. This bond length and bond angle from simulation is shown in Table 1.

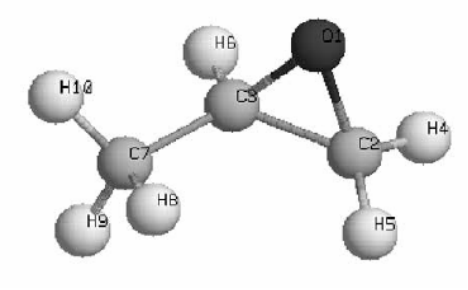

Figure 1 The structures of ethylene oxide and propylene oxide in balls and stick format

Source: Frimand and Jalkanen, (2002).

Table 1 The optimised structure of propylene oxide for the six levels of theory MP2, RHF, DFT/B3LYP, AM1, PM3 and SCC-TB together with the experimental structure

	6-31G*							6-311++G(2d,2p)		
	MP2	RHF	B3LYP	SS-TB	AM1	PM3	EXP	MP2	RHF	B3LYP
r(C2-O1)	1.44	1.40	1.43	1.45	1.43	1.43	1.44	1.44	1.40	1.43
r(C3-O1)	1.44	1.40	1.44	1.46	1.44	1.44	1.45	1.45	1.40	1.44
r(C2-C3)	1.47	1.45	1.47	1.49	1.49	1.49	1.08	1.08	1.45	1.47
r(C2-H4)	1.09	1.08	1.09	1.11	1.10	1.10	1.08	1.08	1.07	1.08
r(C2-H5)	1.09	1.08	1.09	1.11	1.10	1.10	1.08	1.08	1.08	1.08
r(C3-H6)	1.09	1.51	1.09	1.51	1.10	1.11	1.50	1.50	1.50	1.50
r(C7-H8)	1.50	1.08	1.09	1.10	1.49	1.50	1.09	1.09	1.08	1.09
r(H7-H9)	1.09	1.09	1.09	1.10	1.12	1.10	1.09	1.09	1.09	1.09
r(C7-H10)	1.09	1.09	1.10	1.10	1.12	1.10	1.09	1.09	1.09	1.09
θ(H4-C2-O1)	115.2	115.2	115.5	114.9	114.5	116.1	112.0	114.7	115.1	115.1
θ(H5-C2-O1)	115.0	115.4	115.3	114.9	114.6	116.4	112.0	114.6	115.1	115.0
θ(H6-C3-O1)	115.2	113.4	113.4	113.7	113.4	114.7	112.0	113.1	113.3	113.0
θ(C7-C3-O1)	113.2	116.5	116.6	116.2	116.1	117.2	115.1	115.9	116.8	116.8
θ(H4-C2-C3)	116.0	120.3	120.2	119.9	120.6	121.5	119.1	119.8	120.1	120.0
θ(H5-C2-C3)	120.1	119.8	119.3	119.9	120.8	121.7	119.1	119.1	119.6	119.2
θ(H6-C3-C2)	119.2	117.4	117.2	118.1	119.5	119.9	119.1	117.1	117.2	119.5
θ(C7-C3-C2)	117.3	122.5	122.4	121.0	121.5	121.1	122.7	121.5	120.6	122.5
θ(H8-C7-C3)	109.9	110.6	110.6	110.9	111.1	112.7	109.5	110.0	110.7	110.7
θ(H8-C7-H9)	108.4	108.2	108.2	108.3	108.3	107.4	109.5	108.5	108.3	108.3
τ(H8-C7-C3-O1)	-43.9	-43.4	-44.2	-40.8	-40.8	-41.7	-43.0	-43.0	-43.4	-43.5

Distances, r, are measured in Å(Angstroms), angles θ and τ in degrees.

Source: Frimand and Jalkanen, (2002).

From this study, the ab initio method (B3LYP, RHF and MP2) can optimize better than semi-empirical method (AM1, PM3 and SCC-TB). In semi-empirical method, the method SCC-TB can calculate better than PM3 and AM1 methods. In the *ab initio* method, the MP2 can optimize structure of the propylene oxide better than B3LYP and RHF methods, respectively. The 6-31G* and 6-311++G(2d,2p) basis sets give the nearly bond length and bond angle in optimum structure. However, this method can simulate nearly with experimental data.

From the molecular simulations study, the molecular simulation can optimize the molecule and predict the reaction from molecular energy. From this research, the model of drug in glycol chitosan encapsulation delivery is interest because this form can protect the drug disintegration in solution. The capsule can be made from poly(ethane glycol) chitosan. The poly(ethane glycol) chitosan may help the chitosan and doxorubicin to form micelle structure. This micelle can carry drug to cancer cell.