MATERIALS AND METHODS

This thesis focuses on modeling and simulation. Therefore, computational equipment and software are provided for molecular structure and reaction mechanism. A high efficiency computer is used to determine the geometrical parameters of doxorubicin and glucosamine(ethylene glycol) oligomers structures. The reaction step is calculated from relative molecular energy for the polymer bond breaking in glucosamine(ethylene glycol) oligomers molecule and the micelle formation mechanism of glucosamine(ethylene glycol) and the doxorubicin. The molecular modeling software named GaussViewW and GAUSSIAN 03W will be employed in this work.

Materials

The materials or equipments are shown in the following;

1. High efficiency computers:
   Intel® Xeon™ CPU3.0 GHz 2 processors, 2.0 GB of RAM
   Intel® Pentium® 4 CPU 3.00 GHz processor, 2.0 GB of RAM
   AMD Athlon™ XP 64 bit CPU 2.0 GHz processor, 1 GB of RAM
   AMD Athlon™ XP 64 bit CPU 2.0 GHz processor, 1 GB of RAM
   AMD Athlon™ XP 64 bit CPU 2.0 GHz processor, 1 GB of RAM
   AMD Athlon™ XP 2400+ CPU 2.0 GHz processor, 1.5 GB of RAM
   AMD Athlon™ XP 2200+ CPU 2.0 GHz processor, 256 MB of RAM
   AMD Athlon™ XP 64 bit CPU 2.0 GHz processor, 256 MB of RAM

2. GAUSSIAN 03W and GaussViewW 3.09 for windows software.

Methods

Molecular modeling is the specific method to determine any molecular structures and reaction mechanism. This technic can estimate the geometrical parameters in the molecular level such as bond length, bond angle and charge. These parameters can be used to calculate the molecular energy. This molecular energy can estimate the possibility of reaction by relative energy between product and reactant.

Geometrical Parameters Estimations

The geometry of molecules is constructed by GaussViewW. For the simulation, the GAUSSIAN 03W is used to predict sufficient properties of molecules and reactions. A stable molecular structure is illustrated by the minimum molecular energy. In this thesis, geometrical parameters of molecules are determined in the gas phase calculation because simulation in liquid phase has to use higher computer efficiency. The input structure of molecule is calculated by GAUSSIAN 03W to optimize the molecular structure. The geometry optimization structure of the doxorubicin and glucosamine(ethylene glycol) can be described by the optimum bond lengths and bond angles of molecular structure.

The geometrical molecular structure of doxorubicin, glucosamine(ethylene glycol) and glucosamine(ethylene glycol) oligomers are shown in Figures 2, 3, and 4, respectively. Where, the n is amount of ethylene glycol molecule in glycol chain and the m is amount of glucosamine(ethylene glycol) monomers. The structure of these chemicals will be simulated by the semi-empirical AM1, PM3 and *ab initio* Hartree-Fock basis set 6-31G, and then, these structures will be optimized to obtain the most stable molecule.

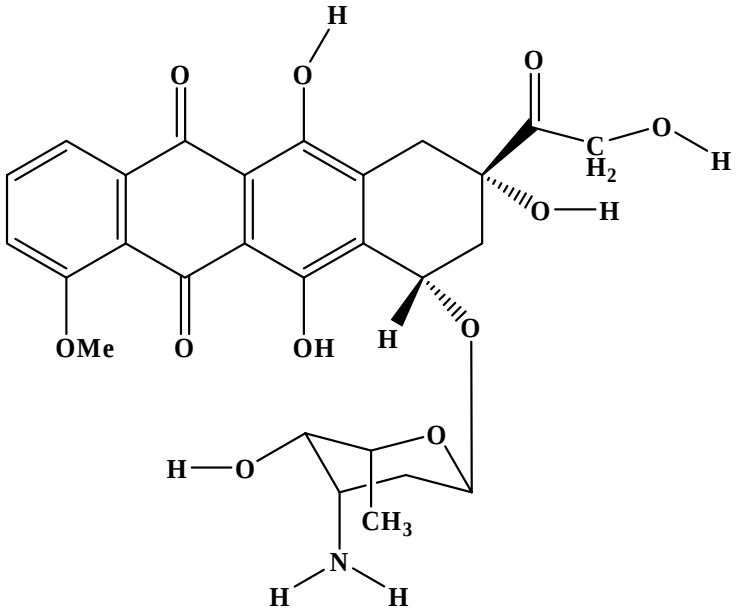


Figure 2 Molecular structure of Doxorubicin

Source: Furgeson, *et al*. (2006)

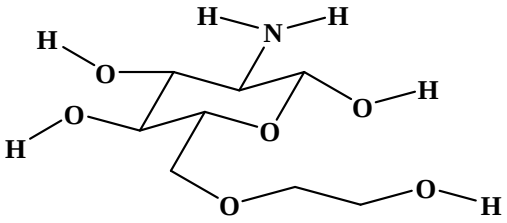


Figure 3 Molecular structure of glucosamine(ethylene glycol)

Source: Modified from Son *et al*. (2003)

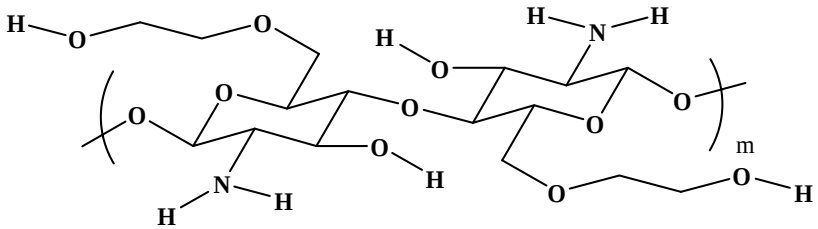


Figure 4 Molecular structure of glucosamine(ethylene glycol) oligomers

Source: Modified from Son *et al*. (2003)

Drug release from capsule

There are two steps for the release of drug from capsule polymer starting with the breaking of polymer bond in capsule, the following step is the drug release. Generally, the rate of bond breaking of capsule polymer is slower than the rate of drug release (Arifin *et al*., 2006). Drug release from capsule depends on the rate of bond breaking of polymer. In this study, the drug release will be simulated from bond breaking of capsule because the polymer bond breaking relates with drug release. If the polymer bond breaking is easy to occur, the drug release rate is high, too.

Doxorubicin or drug in this study can be released from glucosamine(ethylene glycol) oligomers by the simulation of their bond breaking. This simulation will study the effect of solution on breaking bond of di glucosamine(ethylene glycol). The acid-base solution effect for bond breaking is calculated between monomer bonding of glucosamine(ethylene glycol) oligomers by molecular modeling simulation. There are three environments to simulate. In the acid solution, the effect of hydronium ion (H_3O^+) to glucosamine(ethylene glycol) oligomers will be studied. The effect of hydroxide ion (OH^-) can be simulated in base solution. For the normal solution, this simulation will focus on the effect of water (H_2O) to glucosamine(ethylene glycol) oligomers.

The simulations of bond breaking of glucosamine(ethylene glycol) oligomers in solution effect are shown in Figures 5, 6 and 7. The possibility of solution effect can be calculated from relative energy between products and reactants of these reactions.

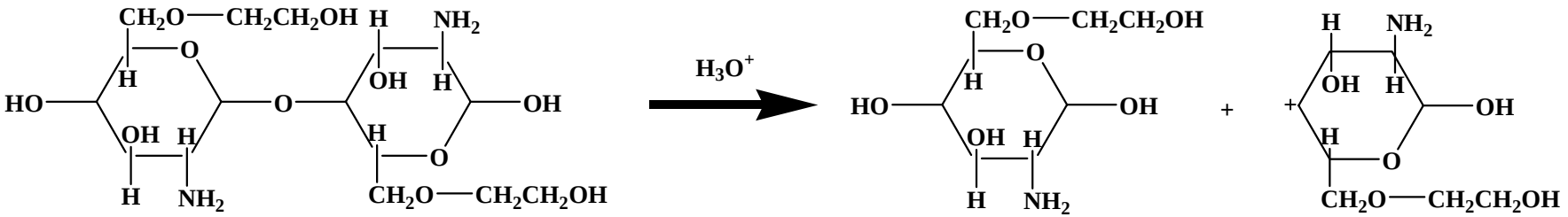


Figure 5 Reaction of acid solution to di glucosamine(ethylene glycol)

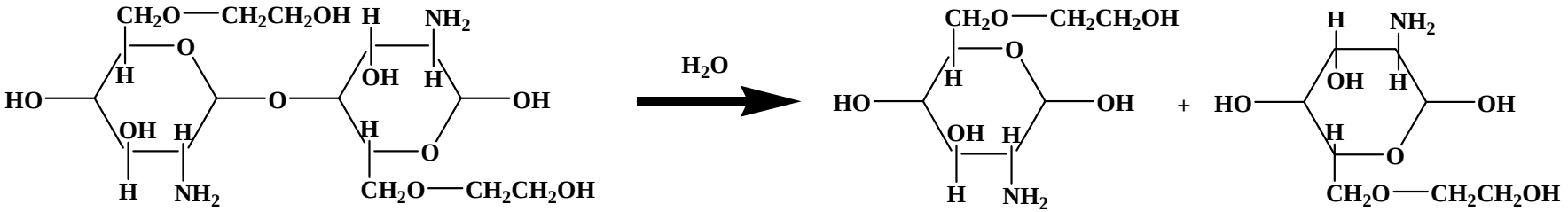


Figure 6 Reaction of water solution to di glucosamine(ethylene glycol)

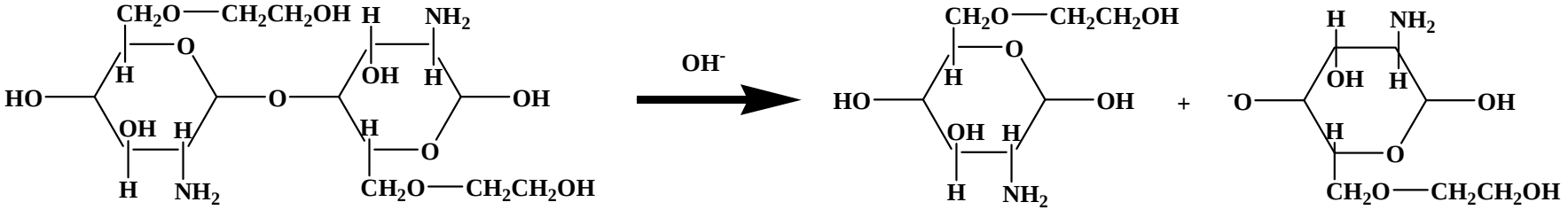


Figure 7 Reaction of base solution to di glucosamine(ethylene glycol)

Micelle formation

After drugs released from polymer capsule, their molecules will be formed the micelle structure with glucosamine(ethylene glycol) because of H-bonds between doxorubicin and glucosamine(ethylene glycol). According to Janes *et al*., (2001), doxorubicin cannot dissolve in the polar solution. The micelle formation of doxorubicin and glucosamine(ethylene glycol) is drug delivery. In this study, micelle formation of doxorubicin and glucosamine(ethylene glycol) can bind from charge of oxygen, nitrogen and hydrogen atoms in their molecules. The charge relates with density of electron.

The micelle formation depends upon amount of ethylene glycol in the glucosamine(ethylene glycol), position of formation, and charge structure in glucosamine(ethylene glycol) and doxorubicin molecules.

Effect of amount of ethylene glycol in the glucosamine(ethylene glycol)

The ethylene glycol can be used as a drug carrier to target of cancer cell (Chan *et al*., 2007). This group carries drug in micelle form by glucosamine(ethylene glycol). The micelle formation depends on the amount of ethylene glycol chain. Therefore, the amount of ethylene glycol promotes the drug carrier and also delivery to the target. Anyhow, the ethylene glycol chain might have stearic effect to doxorubicin releasing from capsule glucosamine(ethylene glycol). Then, the length of ethylene glycol chain in glucosamine(ethylene glycol) might help micelle formation. But, the length of ethylene glycol chain blocks polymer bond breaking of glucosamine(ethylene glycol) oligomers in solution. Then, the length of ethylene glycol chain is not too long to block polymer bond breaking of glucosamine(ethylene glycol) oligomers. The molecule of ethylene glycol is shown in Figure 8.

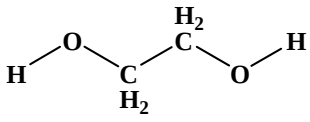


Figure 8 Molecular structure of ethylene glycol

The glucosamine(ethylene glycol) and glucosamine(ethylene glycol) olgomers are shown in Figures 9 and 10, respectively. Where, the n is amount of ethylene glycol molecule in glycol chain and the m is amount of glucosamine(ethylene glycol) monomers.

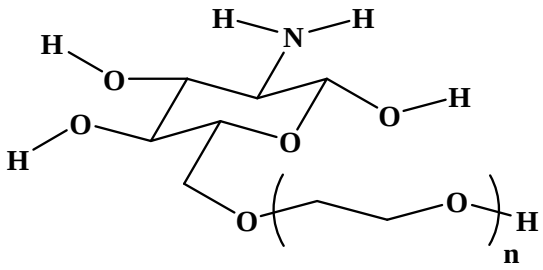


Figure 9 Molecular structure of glucosamine(ethylene glycol)
Source: Modify from Son, *et al.* (2006)

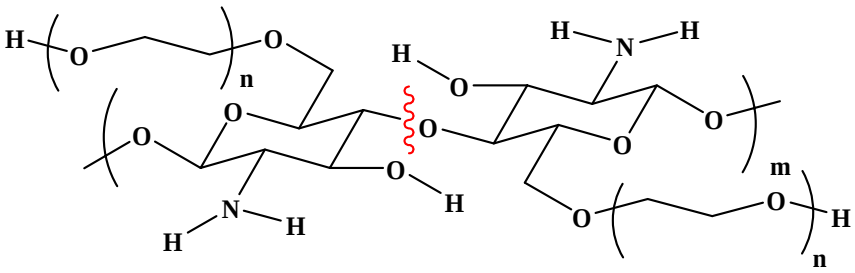


Figure 10 Position of breaking bond of glucosamine(ethylene glycol) oligomers by varying amount of ethylene glycol
Source: Modify from Son, *et al.* (2006)

From Figure 10, if di glucosamine(ethylene glycol) can break at polymer bond, the product of this reaction becomes the glucosamine(ethylene glycol) which has trend to micelle formation with doxorubicin to drug delivery.

Micelle formation mechanism

The mono glucosamine(ethylene glycol) is generated from polymer bond breaking of glucosamine(ethylene glycol) oligomers, and then the doxorubicin releases form this capsule. After that, the molecule of doxorubicin and glucosamine(ethylene glycol), broken form solution attraction, can form by using H-bond to the micelle. The micelle form can perform like drug delivery which the ethylene glycol carries the drug in micelle group to the cancer cell. The H-bond formation is conceived from oxygen, nitrogen and hydrogen of molecule of glucosamine(ethylene glycol) linked to doxorubicin.

The micelle formation of doxorubicin with glucosamine(ethylene glycol) depends on the amount of glucosamine(ethylene glycol) and ethylene glycol chain in glucosamine(ethylene glycol). This work studies the effect of ethylene glycol chain to micelle formation. This effect is simulated by varying the amount of the ethylene glycol in glucosamine(ethylene glycol) from monomer, dimer, and trimer, respectively. For poly(ethylene glycol), this simulation ethylene glycol in pentamer and varies molecule of glucosamine(ethylene glycol) to 1, 2, and 3, respectively. This micelle formations are simulated by B3LYP6-31G//PM3 method. The micelle molecule for simulation is shown in Figures 11, 12, and 13, respectively.

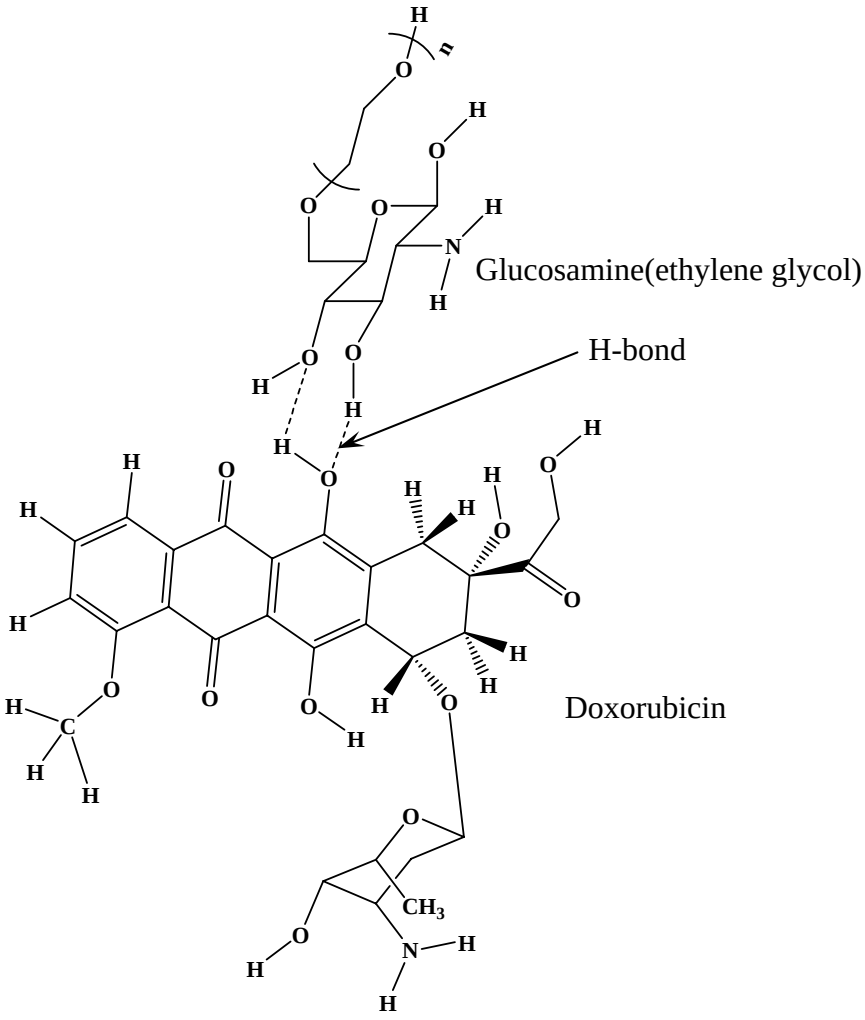


Figure 11 Micelle formations from doxorubicin and glucosamine(ethylene glycol) 1 molecule

Where n = 1; glucosamine-mono(ethylene glycol)

n = 2; glucosamine-di(ethylene glycol)

n = 3; glucosamine-tri(ethylene glycol)

n = 4; glucosamine-tetra(ethylene glycol)

n = 5; glucosamine-penta(ethylene glycol)

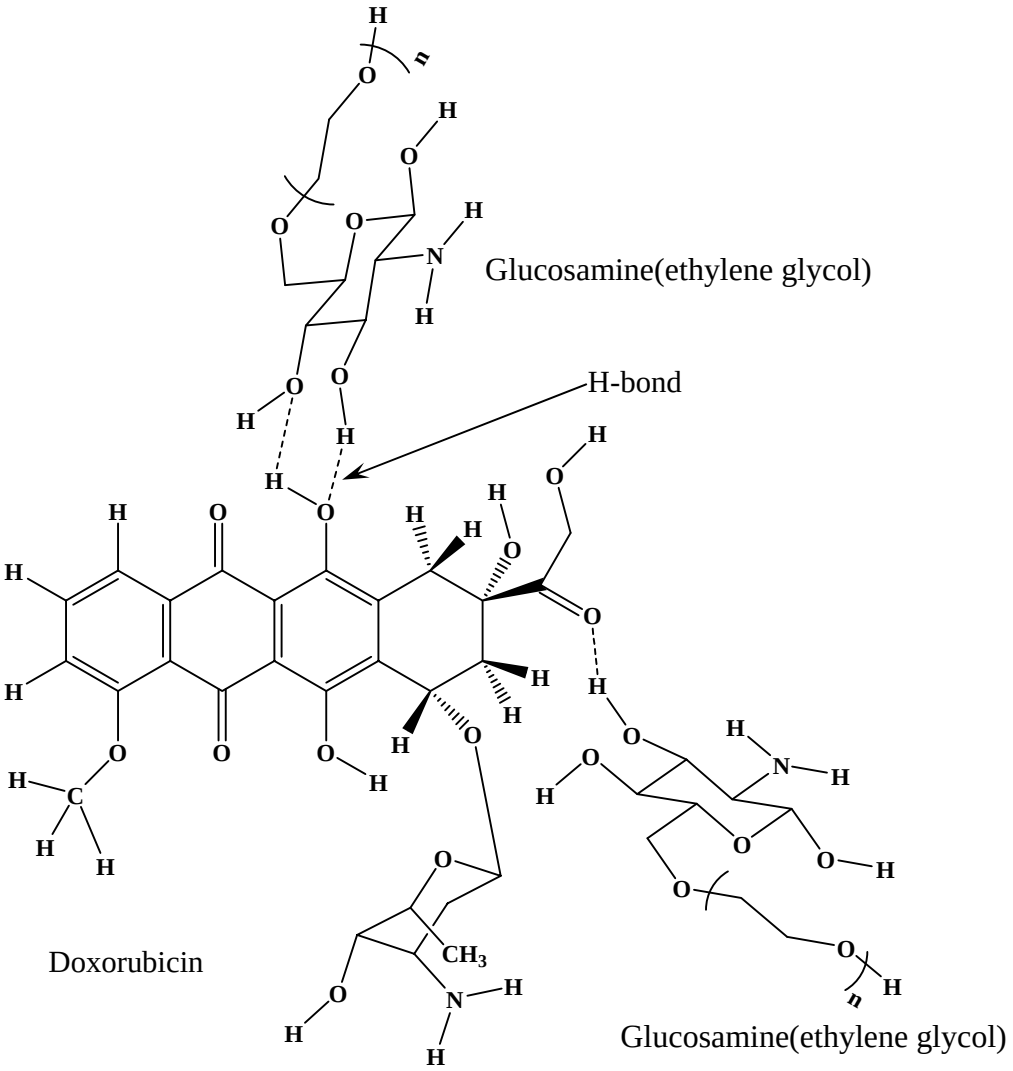


Figure 12 Micelle formations from doxorubicin and glucosamine(ethylene glycol) 2 molecules

Where n = 1; glucosamine-mono(ethylene glycol)

n = 2; glucosamine-di(ethylene glycol)

n = 3; glucosamine-tri(ethylene glycol)

n = 4; glucosamine-tetra(ethylene glycol)

n = 5; glucosamine-penta(ethylene glycol)

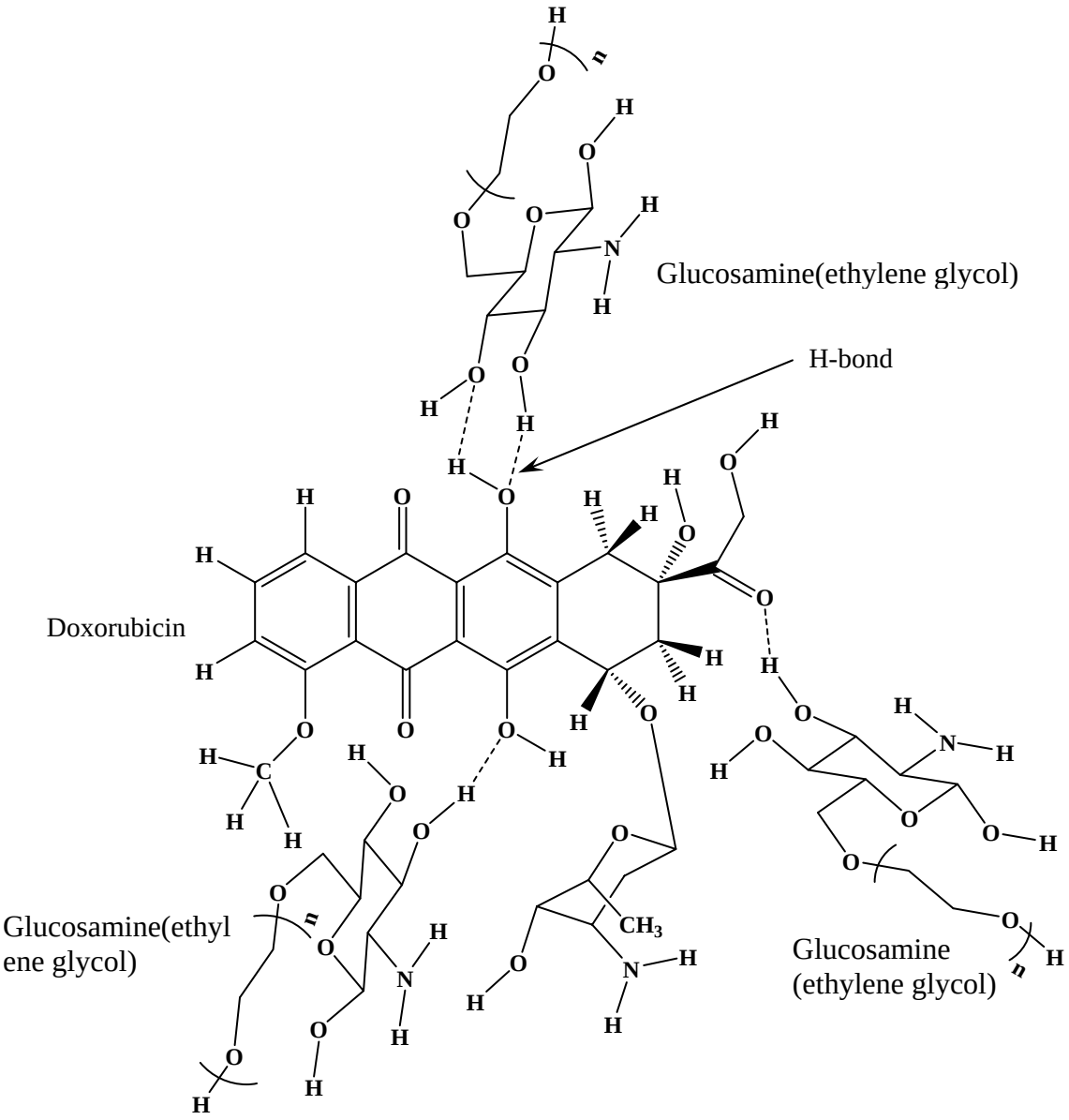


Figure 13 Micelle formations from doxorubicin and glucosamine(ethylene glycol) 3 molecules

Where n = 1; glucosamine-mono(ethylene glycol)

n = 2; glucosamine-di(ethylene glycol)

n = 3; glucosamine-tri(ethylene glycol)

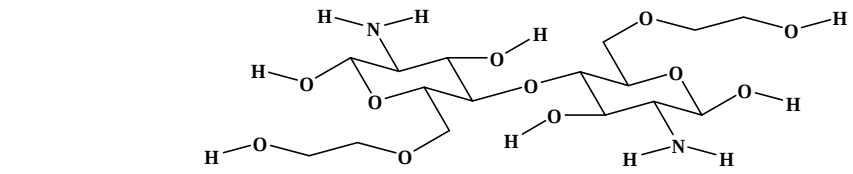
n = 4; glucosamine-tetra(ethylene glycol)

n = 5; glucosamine-penta(ethylene glycol)

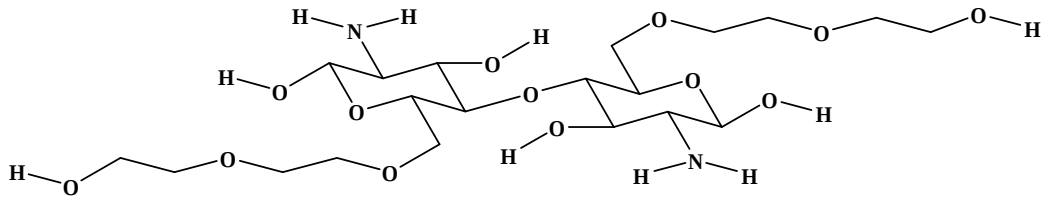
Effect of ethylene glycol to polymer bond breaking of glucosamine(ethylene glycol)

The ethylene glycol chain has an influence to the glucosamine(ethylene glycol) and doxorubicin in order to form micelle structure. Basically, the longer chain of ethylene glycol, the easier micelle can be. On the other hand, this amount of glycol group might affect the glucosamine(ethylene glycol) bond breaking. The effect of glucosamine(ethylene glycol) chain to release drug was studied by comparison between di glucosamine-mono(ethylene glycol) and di glucosamine-di(ethylene glycol) in the solution. The solution contains three conditions; as acid, normal, and base solution. In acid condition, the simulation used H_3O^+ interaction because this molecule is active in acid solution. The normal condition used H_2O. And the base condition, the simulation used OH^- interaction because this molecule is active in base solution.

The molecules of di glucosamine-mono(ethylene glycol) and di glucosamine-di(ethylene glycol) are shown in Figure 14. These molecules are studied an effect of ethylene glycol chain on polymer bond breaking of di glucosamine(ethylene glycol) in order to using in capsulation to protect and carrier doxorubicin.



(a)



(b)

Figure 14 The molecular structure; (a) di glucosamine-mono(ethylene glycol)
(b) di glucosamine-di(ethylene glycol)