CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Magnetite Nanoparticles

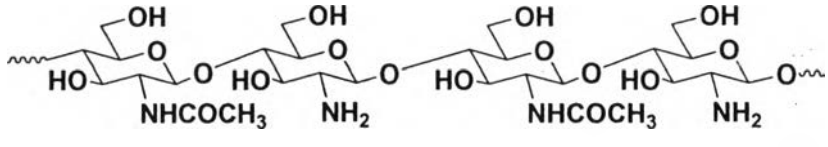
Magnetite, Fe_3O_4, has an inverse spinel structure. The magnetic nanoparticles are magnetite prepared in nano-sized particulate which are accepted for biomedical and pharmaceutical applications such as drug delivery system, hyperthermia, magnetic resonance imaging (MRI), and DNA separation due to their superparamagneticity, together with biocompatibility, stability under ambient conditions, and simplicity of preparation (Pankhurst *et al.*, 2003). The magnetite nanoparticles have been synthesized from the two main approaches for examples, physical approach such as gas phase deposition and electron beam lithography, and wet chemical approach such as co-precipitation. The wet chemical approach is simpler, more tractable, and more efficient than others due to controllable size, composition and sometimes the shape of the nanoparticles (Gupta *et al.*, 2004; Charles *et al.*, 1992).

Magnetite nanoparticles can be synthesized by co-precipitating of Fe^{2+} and Fe^{3+} aqueous salt solutions in a base (Reimers *et al.*, 1972). The overall reaction is $Fe^{2+} + 2Fe^{3+} \rightarrow Fe_3O_4 + 4H_2O$. Besides, Fe_3O_4 might be also oxidized as $Fe_3O_4 + 0.25O_2 + 4.5H_2O \rightarrow 3Fe(OH)_3$ The magnetite nanoparticles can be prevented from oxidation in air as well as from agglomeration by coating with organic or inorganic molecules during precipitation process. In the case of organic molecules, coating of nanoparticles can be done by using (i) polymeric materials such as poly(ethylene-*co*-vinyl acetate), poly(vinylpyrrolidone) (PVP), poly(lactic-*co*-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), poly(vinylalcohol) (PVA), gelatin, pullulan, and chitosan (Schwick, *et al.*, 1969; Akiyoshi *et al.*, 1996; Li *et al.*, 1997; Jeong *et al.*, 1999; Massia *et al.*, 2000), (ii) surfactants such as sodium oleate, dodecylamine, and sodium carboxymethylcellulose to enhance dispersibility in an aqueous media (Denizot *et al.*, 1999; Dresco *et al.*, 1999; Vladimir *et al.*, 1999).

2.2 Chitosan

Chitosan is a linear polysaccharide composing of β-(1,4)-linked *D*-glucosamine (deacetylated unit) and *N*-acetyl-*D*-glucosamine (acetylated unit) (Scheme 2.1). Chitosan is obtained by deacetylating chitin which is collected from skeletons of crustaceans (crabs, shrimps, etc.) and cell walls of fungi. Chitosan has been used in many applications such as food processing, environmental procurement, etc., (Savant *et al.*, 1995) based on its biodegradability, biocompatibility and low toxicity (Borchard *et al.*, 2001; Karlsen *et al.*, 1991). Chitosan becomes an important material when the more developed. Based on the chemical structure, the fact that chitosan has functional groups such as hydroxyl and amino groups, various derivatizations for desired applications are possible.

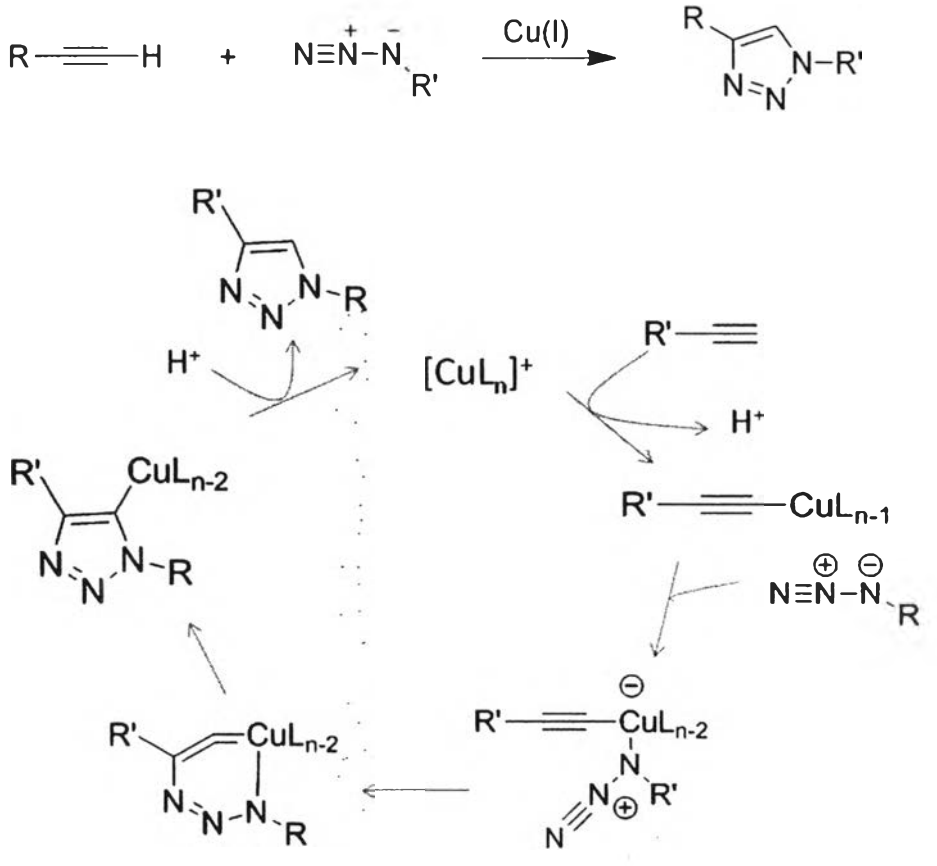
Scheme 2.1



2.3 "Click" Chemistry

The Copper(I)-catalyzed azide-alkyne cycloaddition "click" reaction was introduced by Sharpless (Rostovtsev *et al.*, 2002), and Meldal (TornØe *et al.*, 2002) independently in 2002. It becomes an important surface modification for polymers and other materials, especially in the cases of nanoparticles or other substrates. The "click" reaction is also used for functionalization of polymers in solutions (Fournier *et al.*, 2007). Both alkyne (R-C≡CH) and azide ($R-N_3$) groups are inert for most reactive moieties/functional groups but they combine to generate triazole linkages in the presence of Cu(I) (Scheme 2.2).

Scheme 2.2



"click" chemistry receives much attention in the development of polymer-based bioconjugates (Lutz *et al.*, 2008; Dirks *et al.*, 2007) since it delivers synthetic polymer without complicated reaction steps and gives the compounds with potential biological functions of proteins, polysaccharides, and DNA. It is known that copper has toxicity if it contains in biomaterials (Opsteen *et al.*, 2007). As a consequence the uses *in vivo* and *in vitro* have to be considered. Therefore, many nontoxic copper-free "click" reactions were developed in the field of chemical biology in recent years (Lutz, *et al.*, 2008). The important copper-free "click" procedures for both synthesis and functionalization of polymeric coating are important for biological active materials (Canalle, *et al.*, 2009).

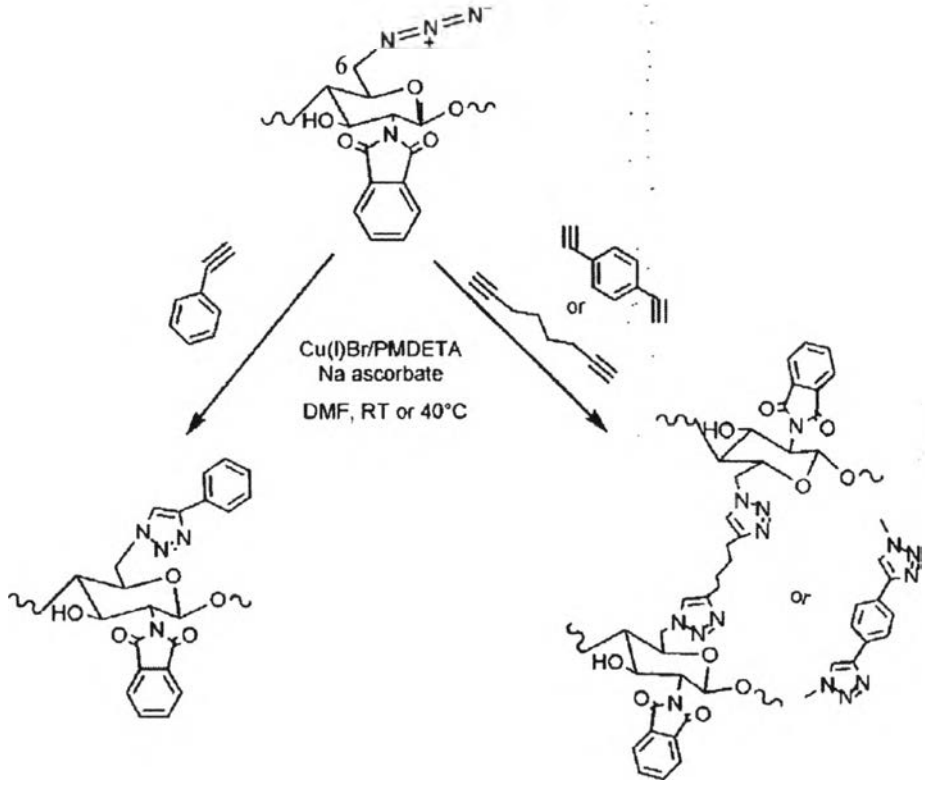
The 1,2,3-triazole obtained from the "click" reaction is an additional functional group giving hydrogen bond and coordination. Moreover, the triazole group possesses a broad spectrum of biological properties, not only anti-HIV, anti-

allergenic and antibacterial features but also fungicidal and herbicidal activity (Krasinski *et al.*, 2004).

2.3.1 "Click" Chemistry with Chitosan

Chitosan structure can be modified to introduce azido groups at C-6 position by coupling with mono- or di-alkynes by using Cu(I) catalyzed dipolar cycloaddition as shown in Scheme 2.3 (Zampano, *et al.*, 2010).

Scheme 2.3

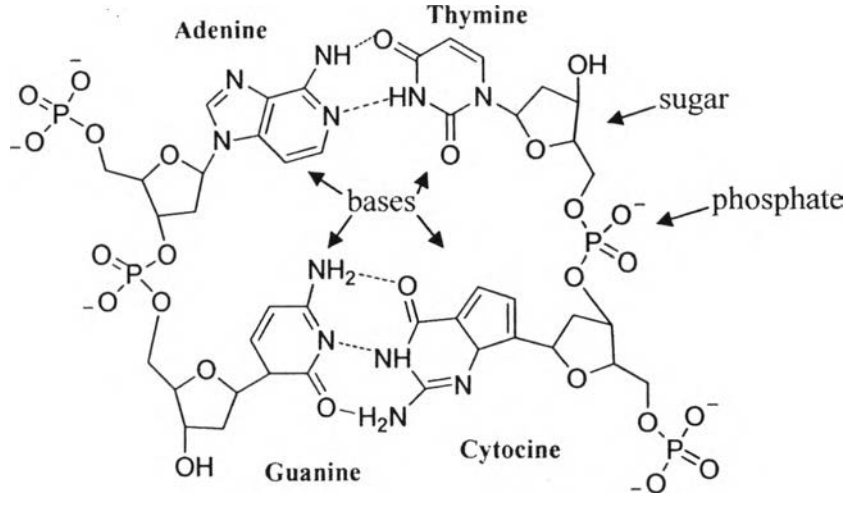


2.4 DNA Separation

DNA separation is an important method to isolate DNA from samples such as blood, cells, etc., for further specific research including personal identification and defining disease. In the past, phenol extraction and ethanol precipitation were applied for DNA separation but these methods consumed a long test time, toxic agent, and

complicated (Powell *et al.*, 2002). Moreover, there are several available methods for example, (i) binding plasmid to silica in the presence of high concentrations of chaotropic salts (Chen and Thomas, 1980; Marko *et al.*, 1982; Boom *et al.*, 1990), (ii) differential precipitation of plasmid DNA from aqueous chaotropic salt/ethanol solutions (Hamaguchi and Geiduschek, 1962; Wilcockson, 1973; Wilcockson, 1975) and ion exchange chromatography over DEAE-modified cellulose membranes (Huynh *et al.*, 1993) and (iii) precipitation with polyethylene glycol (Lis, 1980; Paithankar and Prasad, 1991). DNA structure (Scheme 2.4), which consists of sugars, bases, and phosphates is unique in terms of it provides hydrogen bond, stacking conformation for DNA helical structure, and the negative charges on the surface to bind with positive polymers.

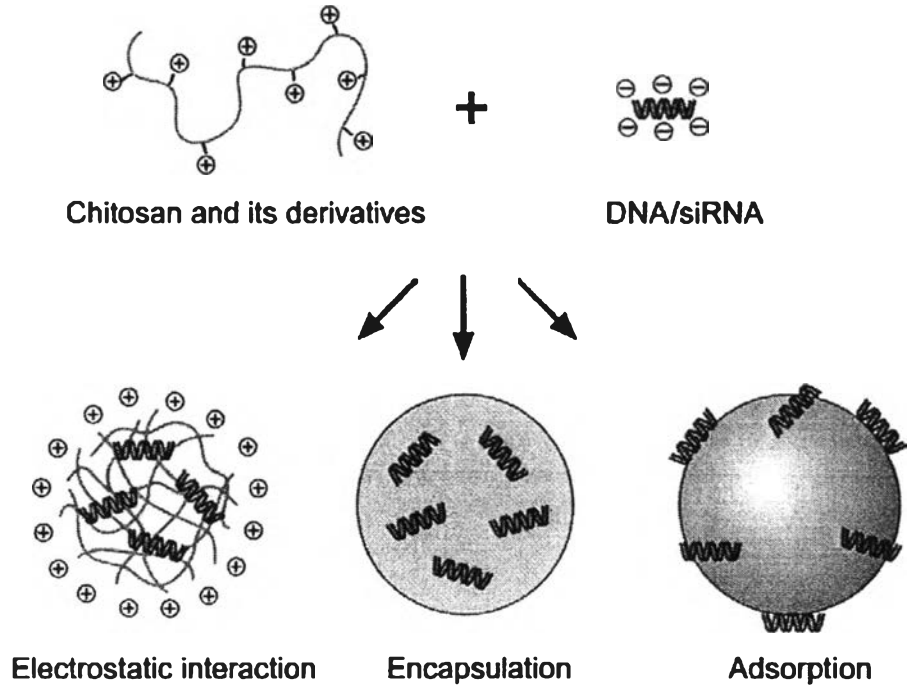
Scheme 2.4



2.4.1 DNA with Chitosan

Chitosan is a cationic aminopolysaccharide which provides positive charges through amino groups whereas DNA covers with negative charge through phosphate groups. Thus, theoretically, both polymers form complexes via electrostatic interaction, encapsulation, and adsorption as reported by Mao *et al.*, 2010 (Scheme 2.5).

Scheme 2.5



2.5 Points of the Present Work

Considering chitosan-magnetite nanoparticles, the key point is how to conjugate chitosan with magnetite nanoparticles via covalent bond. This work proposes the use of "click" chemistry to obtain chitosan-magnetite nanoparticles. Here the approach in consideration consists of three steps, (i) modifying chitosan with alkyne group, (ii) modifying surface of magnetite nanoparticles with silane coupling agent followed by introducing azide group at terminal group and (iii) conjugating (i) and (ii) via "click" chemistry in the condition without copper catalyst. The work also extends to preliminary study on plasmid DNA (pDNA) separation.