CHAPTER I

INTRODUCTION

1.1 Concept of supramolecular chemistry

Supramolecular chemistry has been defined by Prof. Lehn in 1987, as the 'chemistry of molecular assemblies and of the intermolecular bond', which illustrates the term of supramoleculars are composed of two or more molecules held together by electrostatic forces, by hydrogen bonding, by coordination, by hydrophobic, by van der Waals attractive forces and by pi-pi interactions [1]. Important concepts that have been demonstrated by supramolecular chemistry including molecular self-assembly, folding, molecular recognition, host-guest chemistry, mechanically-interlocked molecular architectures, and dynamic covalent chemistry [2]. Supramolecular chemistry is classified into three categories: (i) the chemistry associated with a molecule recognizing a partner molecule (molecular recognition chemistry); (ii) the chemistry of molecules built to specific shapes; (iii) the chemistry of molecular assembly of numerous molecules [3]. Actually, molecular recognition has been studied more widely than the others.
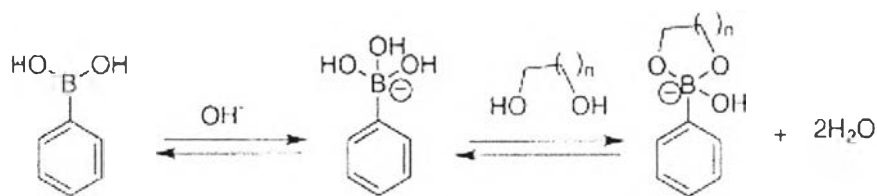
Molecular recognition has been defined as a process involving both binding and selection of substrates by a given receptor molecule, as well as possibly a specific function. The binding is not recognized, although it is often taken as such. The perfect recognition between substrates and receptors was affected by many factors such as geometry, electronic and polarity of substrates and receptors [1]. Studies of molecular recognition require suitable molecular receptors to specifically interact with many substrates.
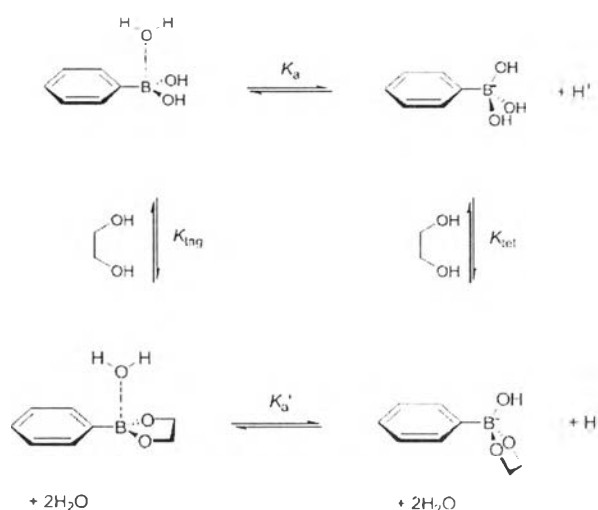
1.2 The complexation between boronic acid based sensor and saccharides

Saccharides are biologically significant molecule which is involved in many cellular processes. The multifunction of them is directly related to pathological processes, cancer [4-6], immune responses [7], and viral [8-12]. Therefore, the identification and detection of the saccharides in the bloodstream are growing importance for diagnosis of human diseases. Many years ago, boronic acid has been used as the key recognition moieties in the construction of sensor for saccharides because of the ability of boronic acid to bind with compounds containing diol moieties with a high affinity through the reversible ester formation (Scheme 1.1). Owing to the possibility of formation of parent anions in aqueous media, several equilibrium reactions are possible. Boronic acid has a 120° (sp^2) bond angle but on the formation of a cyclic ester, the bond angle is reduced to 108° (sp^3). Obviously, the compression of bond angle from 120° to 108° makes the change in hybridization from sp^2 (trigonal) to sp^3 (tetrahedral) (Scheme 1.2). Typically differences in rate of 10^4 are observed between boron in its trigonal and tetrahedral forms (quartet > k_{tri}) [13]. It is also known that the neutral boronic acid becomes more acidic upon binding, in other words, the boronic ester is more acidic than the boronic acid (pK_a > pK_a') [14].

Scheme 1.1 Ester formation with boronic acid reaction in aqueous media [14].

Scheme 1.2 Equilibrium for the boronic acid that reacts with diol in aqueous media.

Most of the saccharides sensors containing the boronic acid as binding sites for saccharides have been investigated by fluorescence spectrophotometry. Across the diverse range of boronic acid based fluorescent sensors developed, two distinct design principles predominate in the scientific literature: photoinduced electron transfer (PET) and internal charge transfer (ICT). In both cases successful signalling of the binding to either the bound or unbound sensors, these processes affecting defined the spectral change in the emission band.

1.2.1 Photoinduced Electron Transfer (PET)

Photoinduced electron transfer (PET) has been widely used as the tool in fluorescent sensor design for molecular species [15-20]. PET sensor is accomplished by connecting a receptor and a fluorophore separated by a spacer. From the simplest cases of the emission of fluorescence, the electron from the highest occupied molecular orbital (HOMO) goes to the lowest unoccupied molecular orbital (LUMO) and then the excited electron of the sensor in LUMO returns to HOMO level and emits the fluorescence. Thus, when a lone pair electron is located in an orbital of the fluorophore itself or an adjacent molecule and the energy of the orbital lies between HOMO and LUMO, efficient electron transfer of one electron of the pair to the hole in the HOMO produced light absorption may occur, then the initially excited electron is transfered to the lone pair orbital. These PET gives a mechanism for nonradioactive inactivation of the excited state (see Figure.1.1), resulting in the reduction emission intensity or quenching of fluorescence [19, 21-22].
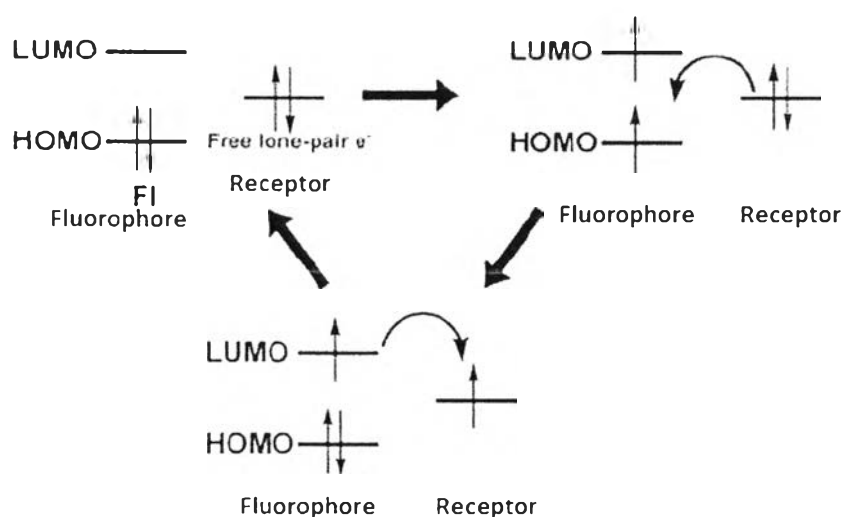
Figure 1.1 The PET process in terms of molecular orbitals of the fluorophore.

1.2.2 Fluorescence sensors for saccharide based on intramolecular charge transfer (ICT)

The intramolecular charge transfer or photoinduced charge transfer phenomenon is usually occurred when a fluorophore contains an electron-donating group conjugated with an electron-withdrawing group upon excitation by light. The consequent difference in dipole moment leads to a Stokes shift that relies on the microenvironments from the fluorophore. Thus, it is usually expected that cations or anions in close connection using the donor or acceptor moiety can change the photophysical properties of the fluorophore [23-25]. After, for instance, cation complexation of the electron donor group with a fluorophore, the electron-donating character from the donor group will be reduced. This resulting decrease in conjugation leads to a new blue shift from the absorption spectrum with a concomitant reduction of the molar absorptivity. On the other hand, metal ion binding with the acceptor group will increase the electron-withdrawing, as well as the spectrum will show a red-shifted absorption band with an increase in molar absorption (**Figure 1.2**) [26]. The fluorescence spectra must be shifted within the same direction because the shift of absorption spectra gives an influence on the excited state of fluorescence. These photophysical effects must be influenced by the charge and also the size of cation, and thus, some selectivity is predicted [27-37].
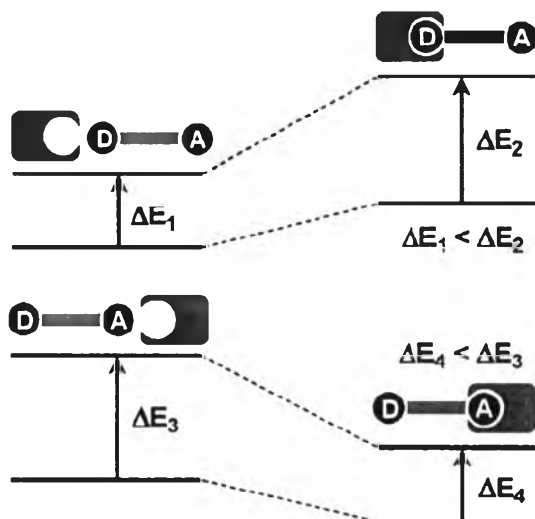
Figure 1.2 PCT process of connecting together with donor group and acceptor group.

1.3 Gold nanoparticles (AuNPs)

General, sensors feature two components: a recognition element to present selective/specific binding with all the aim analytes as well as a transducer component for signaling the binding event. An efficient sensor depends on these two components for that recognition process in relation to response time, signal-to-noise (S/N) ratio, selectivity, and limits of detection (LOD) [38-39]. Therefore, the building sensors having higher efficacy depend upon the development of novel materials to further improve both the recognition and transduction processes. Nanomaterials feature characteristic physicochemical properties which can be of great utility in creating new recognition and transduction processes for chemical and biological sensors [39–51], together with increasing the S/N ratio by miniaturization of the sensor elements [52].

The optical attributes of colloidal gold have been known for a very long time. The most commonly refer and interesting example is the Lycurgus Cup in the 4th century AD: it displays an exceptional green-red dichroism in reflected and transmitted light. Faraday, in the mid-19th century, who began formal investigations in the interaction between gold colloids and light [53]. In the 20th century, physical descriptions, including Mie theory [54] and Gans theory [55] were able to explain and predict the optical attributes of gold nanoparticles. This theory have been widely applied since it allows the computing of the particle quenching spectra, as long as the material's dielectric function is well known and also the size is small compared to the wavelength of the light. The physical origin of the light absorption through metallic nanoparticles is the coherent oscillation on the conduction band electrons induced by the interacting electromagnetic field.

Gold nanoparticles (AuNPs) have unique physical and chemical features which make them excellent scaffolds to the fabrication of new chemical and biological sensors (Figure1.3) [56-63]. Furthermore, we will describe the interesting of properties of AuNPs by initially starting to explain the electronic structure of AuNPs.
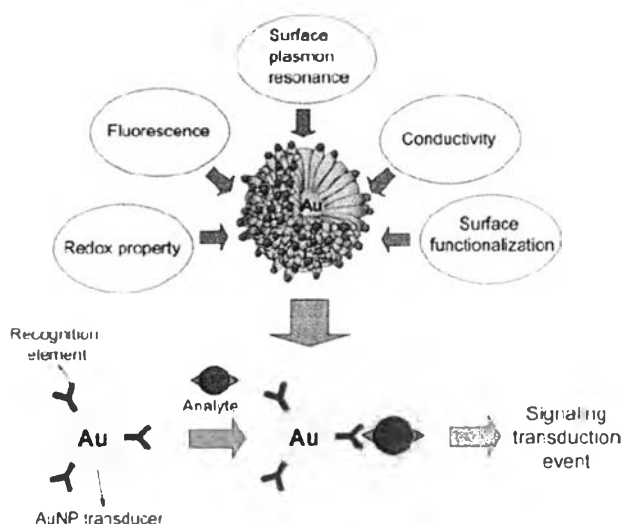
Figure 1.3 Properties of AuNPs and schematic demonstration of a AuNPs-based detection process.

1.3.1 Electronic structure

Gold nanoparticles consist of tens to thousands of gold atoms and thus make an electronic structure that comprises the features of both the discrete levels of energy discovered in atoms and molecules and the band structure discerned in expanded metallic components. Figure 1.4 is usually a qualitative image of the alterations in electronic structure as gold is shrunk from the bulk state, through the nanoscale, and on to the molecular species. In bulk gold, the electronic properties are featured by the valence and conduction bands, consists of an endless number of bonding and anti-bonding orbitals, respectively. The Fermi level is situated inside the conduction band, permitting the metal to conduct only using thermal energy. As the dimensions of the metal are decreased just to a few tens of nanometers, some discrete levels of energy starts to display up at the band borders. Although the properties of the gold nanoparticle stay mostly metallic, some molecular transitions can be discerned under certain conditions, such as the reduced temperature. As the

dimension is further decreased to below 2 nm, the further intercalation of discrete levels of energy inside the band structure is usually discerned. As a result, the nanoparticle loses the most of its metallic features and there is an increased prospect of discerning molecular transitions under ambient conditions.

Further declines in dimensions to small clusters and molecular species view the electronic properties covered with molecular transitions. These alterations in the electronic structure with size emphasise the requirement for getting access to well-defined gold nanoparticles depending on the core diameter [64]. Because smaller gold nanoparticles (< 2 nm) do not have the continuous band structure of large gold nanoparticles and bulk gold, they have intriguing electronic properties which make them of utility in the appearing field of nanoelectronics.
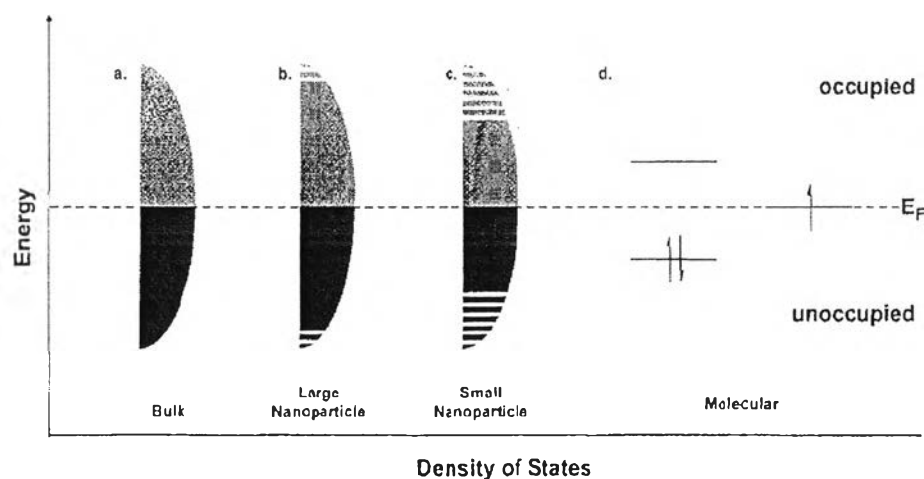

Figure 1.4 The schematic diagram of the density of states for gold transferring from the bulk state towards atomic state [64].

1.3.2 The surface plasmon band (SPB)

The SPB is caused by the collective oscillations of the free electron on the surface of nanoparticles (6s electrons on the conduction band for AuNPs) which is correlated using the electromagnetic field with the incoming light. In addition, with

this size regime AuNPs experience intrinsic size effects: as the shape or size of nanoparticle changes, the surface geometry changes leading to a shift in the electric field density on the surface. This leads to changing the oscillation frequency with electrons, making different cross-sections for the optical attributes, as well as absorption and scattering.
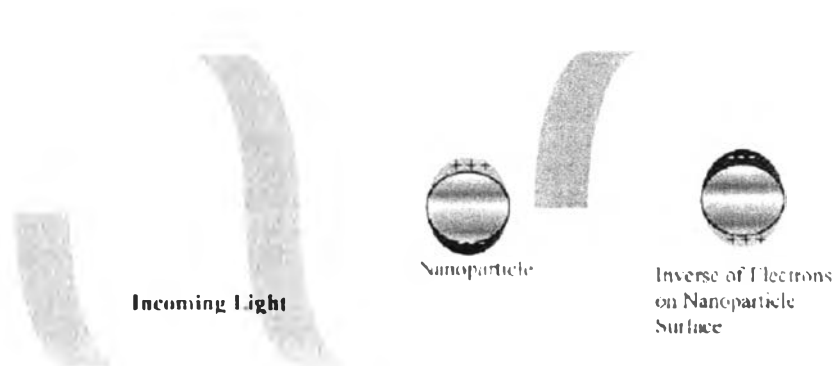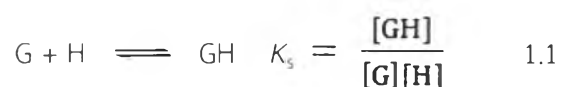

Figure 1.5 Origin of surface plasmon resonance caused by coherent interaction with the electrons on the conduction band with light [65].

1.4 Formation of 1:1 complex [66,67]

$$G + H \rightleftharpoons GH \quad K_s = \frac{[GH]}{[G][H]} \quad 1.1$$

Y_0 would be the fluorescence intensity from the free host. In fluorometric experiments, this absorbance at the excitation wavelength must be lower than 0.1. Y_0 is proportional toward the concentration of the host c_H:

$$Y_0 = a c_H \quad 1.2$$

And the presence of an excessive amount of guest is completely complexes, Y reaches the limiting value Y_{lim};

$$Y_{\text{lim}} = bc_H \qquad 1.3$$

In spectrofluorometry, *a* and *b* are generally proportional towards the molar absorption coefficients with the excitation wavelength and also the fluorescent quantum yields with the host and complex, respectively. After addition the number of guests at the concentration c_G, the fluorescence intensity becomes

$$Y = a[H] + b[GH] \qquad 1.4$$

Mass balance equation with the host and guest are

$$c_H = [H] + [GH] \qquad 1.5$$

$$c_G = [G] + [GH] \qquad 1.6$$

From Equation 1.1 to 1.6, it is possible to derive the normal relation

$$\frac{Y-Y_0}{Y_{lim}-Y} = K_s[G] \qquad 1.7$$

When Y_{lim} is just not measurable because full complexation are not attainable for a reasonable concentration of guest, it is best to utilize this relation:
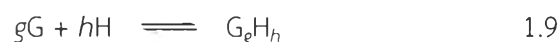
$$\frac{Y_0}{Y-Y_0} = \frac{\alpha}{K_s[G]} + \alpha \qquad 1.8$$

Where α = *a*/(*b-a*). The plot $Y_0/(Y-Y_0)$ compared to $1/c_G$ providing with the approximation [G] ≈ c_G is valid. The ratio from the ordinate for the origin towards the slope yields K_s. This type of plot is widely known as a double-reciprocal plot or a Benesi-Hildebrand plot.

1.5 Determination of the stoichiometry of a complex by the method of continuous variations (Job's method) [68]

Information on the stoichiometry of a complex can also be obtained from the continuous variation method. To calculate a complex G_gH_h formed using the equilibrium

$$gG + hH \rightleftharpoons G_gH_h \qquad 1.9$$

with

$$\beta_{gh} = \frac{[G_gH_h]}{[G]^g [H]^h} \qquad 1.10$$

The basic principle of the method as follows: the fluorescence intensity Y will be measured to get a series of solution composed of the host and the guest such that *the sum of the total concentration of ligand and cation is constant.*

$$C_H + C_G = C = \text{constant} \qquad 1.11$$

The position of the maximum of Y will be related to this ratio *g/h*. The intensity of complex product is equal to Y_0 and also the value of Y relates to no guest added (x=0). When plotting the variations in fluorescence intensity versus X, it is convenient to subtract the fluorescence intensity that will be measured without of guest at each and every concentration.
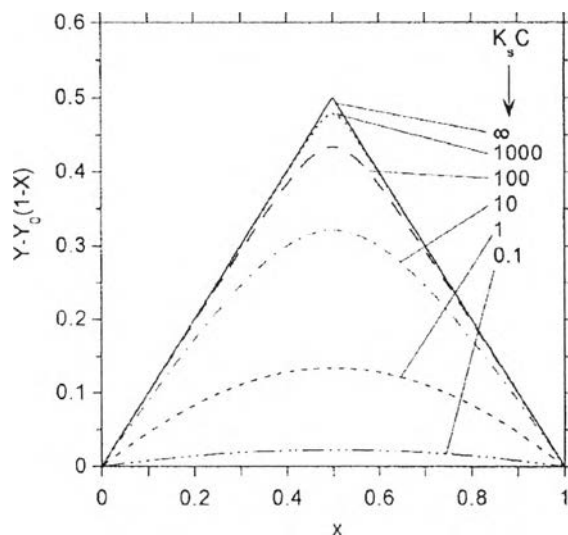
Figure 1.6 Job's plots method for a 1:1 complex

1.6 Limit of detection [69]

A limit of the detection of the analyst is often thought as the concentration giving as instrument signal significant change through the blank or background. This concept of the detection is fairly arbitrary and entirely prepared to take an analyst to offer an alternate definition to get a specific objective. However it is really needed to provide definition if a detection limit is cited within a report.

It is really an alternative definition of which determines the detection limit because the analyte concentration gives a sign equal to the blank signal, y_B, plus three standard deviations with the blank, s_B.

$$\text{Limit of detection} \quad = \quad y_B + 3s_B \qquad\qquad 1.12$$

Based on equation 1, the additional value of the limit of detection, the determined intercept of the calibration plot may be used instead of y.