CHAPTER II
LITERATURE REVIEWS

2.1 Literature reviews

Saccharides play significant role in biological system which is involved in many cellular processes. For instance, saccharides are nature's conveyors of energy and essential for cell survival. Therefore, the identification and detection of the saccharides in bloodstream are growing importance for diagnosis of human diseases. In recent years, a wide variety of sensors for biologically important sugar such as glucose, ribose and fructose has been reported as colorimetric [70], optical rotation [71], near infrared spectroscopy [72], electrochemistry [73] and fluorescence detection [74]. The boronic acid moiety has been known to have high affinity for sugar, high stability and low toxicity. Thus, there is a growing interest in the development of boronic acid derivatives for saccharides sensing based on different measurements employed to design effective sacchride sensors.

2.1.1 Determination of saccharides based on boronic acid

Pina Luis *et al.* [75] presented *m*-dansylaminophenylboronic acid (**DAPB**) as chemosensor for detecting fructose in aqueous solution. Sensor **DAPB** binding with fructose exhibited fluorescence quenching and consequently shifted the pK_a from 8.13 to 7.80 as shown in fig 2.1. This influence could be described taking into account that the boron-nitrogen bound may be strengthened by adding sugar. On the other hand, the photoinduced electron transfer (PET) process could occur through an inverse electron interaction between the n- and π-electronic systems of the complex.

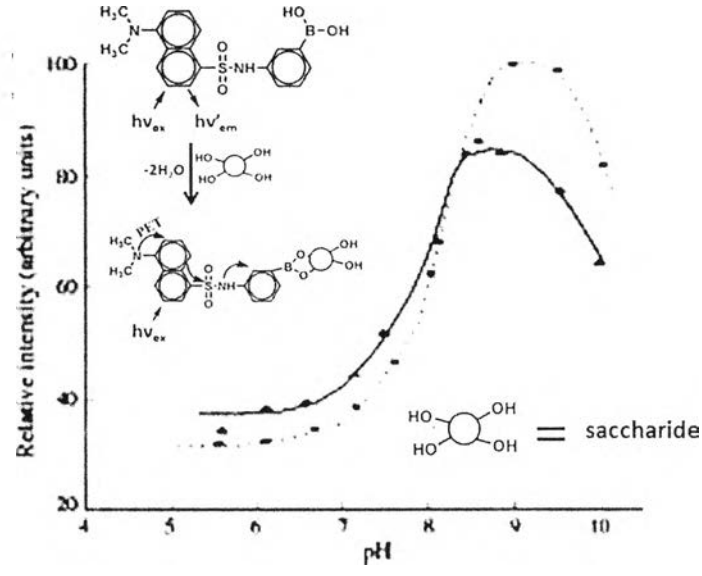

Figure 2.1 Fluorescence intensity versus pH profile of DAPB (1.0×10^{-5} M) in aqueous solution with (▲) and without (■) fructose (1.5×10^{-3} M).

They applied the detection of fructose in food samples as shown in Table 2.1. The results obtained for fructose are consistent with those obtained using an enzymatic kit for food analysis (Boehringer Mannheim).

Table 2.1 Determination of fructose in food

Sample	Fructose found*/g per 100g	
	Proposed method	Enzymic kit
Jam	35.58 ± 1.74	35.80 ± 0.65
Fruit juice	2.71 ± 0.02[†]	2.69 ± 0.02
Whole biscuit	11.29 ± 0.38	11.24 ± 0.49
*Number of analyses ≥ 4. [†]g per ml.		

Arimori *et al.* [76] invented saccharide sensor **1** that consisted of aniline and boronic acid for covalent bond of diol of saccharides by using efficient internal charge transfer (ICT) in Fig 2.2. Sensor **1** was highly selective and sensitive toward fructose through a combination of reversible boronic ester formation. This sensor showed a blue shift of about 42 nm and an increase of fluorescence intensity in emission spectrum upon increment of fructose as shown in Fig 2.3. From Fig 2.3, isosbestic point was observed at 377 nm indicating that sensor **1** changes from a single unbound species to a single saccharide bound species.

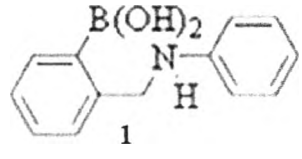

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Figure 2.2 Proposed structures of compound **1**

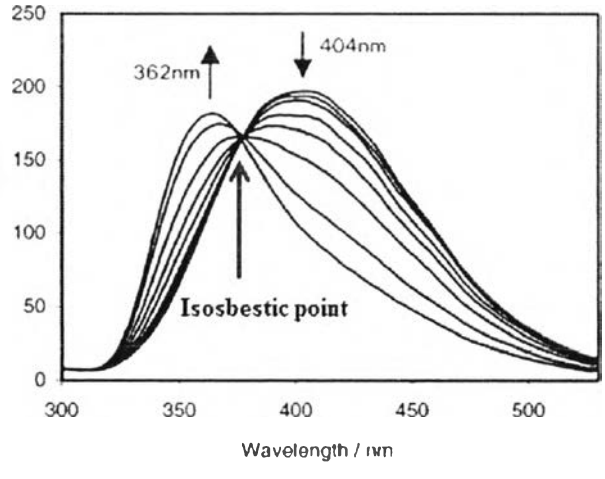

Figure 2.3 Fluorescence spectra of sensor **1** versus different amounts of fructose in MeOH (52.1 wt%) in phosphate buffer at pH 8.21 at λ_{ex}= 274 nm.

This phenomenon was due to loss of electron of boron sensor upon hybridization change from sp^2 to sp^3 and change in the environment of nitrogen atom which leads to change in energy levels of the n and **ΠΠ*** orbital of the sensor [77,78]. Moreover, sensor **1** displayed the stability constants for other analytes in Table 2.2.

Table 2.2 The stability constants, *K* (coefficient of determination; r^2) for saccharide of compound **1**

D-Fructose	D-Glucose	D-Galactose	D-Mannose
106±7 (0.99)	18±6 (0.98)	27±4 (0.99)	–[a]

[a] The *K* could not be determined because of the small fluorescence change.

Hayashita *et al.* [79] designed a new fluorescence probe that consisted of a fluorescent pyrenyl group and arylboronic acid as a binding site for saccharides [80] and β-cyclodextin (β-CyD) as the incorporated probe in terms of the assembled probe **2**/β-CyD as a saccharides sensor (in Fig 2.4).

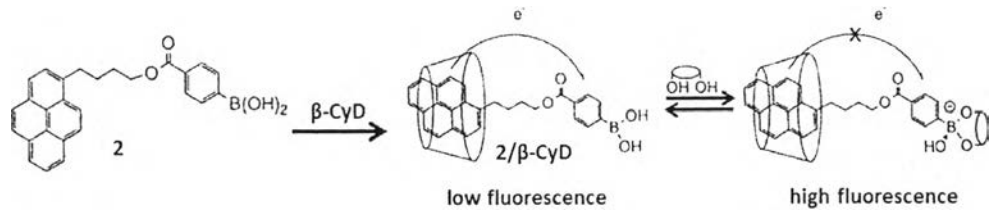

Figure 2.4 Proposed structure of probe **2** and mechanism of **2**/β-CyD complexed with saccharides under PET process.

The absorption and fluorescence spectra of **2** in aqueous solutions with different DMSO contents are shown in fig 2.5. The absorption peaks of **2** in 2% DMSO showed a broad peak when compared to probe **2** in 25% DMSO, indicating that probe **2** aggregated in water and fluorescence spectra of **2** displayed no signal because the boronic acid group has trigonal geometry. The arylboronic acid can act as an electron acceptor from the excited pyrene donor. This results in strongly PET pathway from the pyrene donor to the acid form of the arylboronic acid acceptor [81]. In contrast, probe **2** in 25% DMSO and buffer solution showed fluorescence of monomer emission of pyrene. The addition of β-CyD to probe **2** in 2% DMSO in buffer solution exhibited the absorption peaks similar to that in 25% DMSO in buffer solution and the fluorescence intensity of **2** was enhanced in the presence of β-CyD since β-CyD inhibited free movement of **2** and then reduced the radiationless of the transition process.

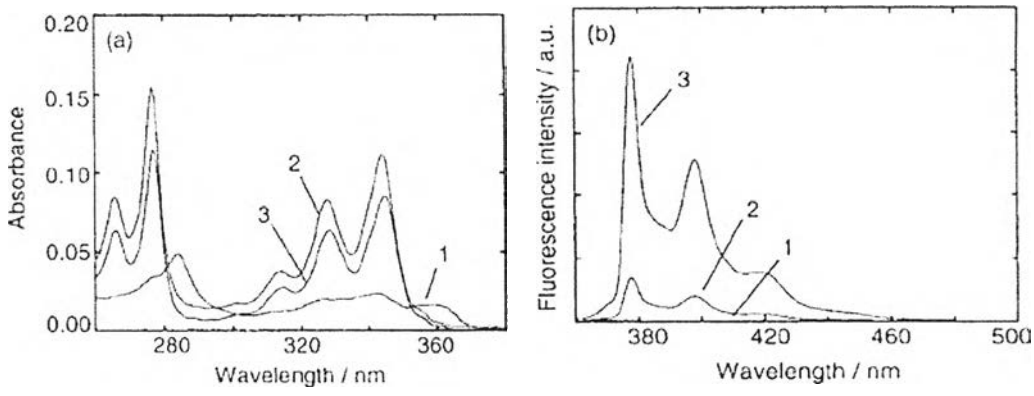

Figure 2.5 a) Absorption spectra of **2** b) fluorescence spectra of **2** at λ_{ex} = 328 nm in DMSO with 0.015 M phosphate buffer at pH 7.5: (1) 2% DMSO/98% buffer solution (v/v), (2) 25% DMSO/75% buffer solution (v/v), (3) 2% DMSO/98% buffer solution (v/v) containing 5.0 mM β-CyD.

The complexation of **2**/β-CyD toward fructose exhibited increase of fluorescence intensity after adding fructose as shown in Fig 2.6 a). This phenomenon can be explained that boronic acid can converted to tetrahedral boronate form induced by saccharides binding. In contrast, probe **2** with fructose in 2% DMSO displayed no fluorescence response (cuve 1 in Fig 2.6 b) and probe **2** that with fructose in 25% DMSO showed increase of monomer fluorescence intensity (cuve 2 in Fig 2.6 b).

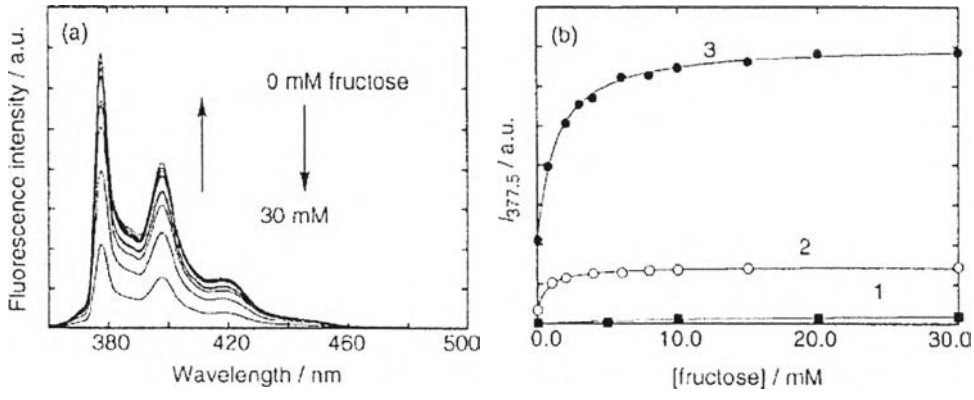

Figure 2.6 a) Fluorescence spectra of **2**/β-CyD with different amounts of fructose in 2% DMSO with 0.015 M phosphate buffer at pH 7.5. b) the relative fluorescence intensity of **2** with concentration of fructose: (1) 2% DMSO/98% buffer solution (v/v), (2) 25% DMSO/75% buffer solution (v/v), (3) 2% DMSO/98% buffer solution (v/v) containing 5.0 mM β-CyD.

Mohr *et al.* [82] synthesized a new napthalimide derivative containing boronic acid group for detecting saccharides in the physiological pH range. This sensor shows a weak emission band due to electron transfer from lone pair electron of nitrogen atom to the fluorophore. Upon adding of saccharide, the ester boronic acid conplexes inhibits the electron transfer resulting in fluorescence enhancement as shows in figure 2.7. They reported that sensor **3** shows the highest recognition toward fructose observing from the association constants as indicated in Table 2.3. The compound **3** toward D-galactose and D-glucose displayed lower binding constant. Therefore, compound **3** showed a high selectivity with fructose.

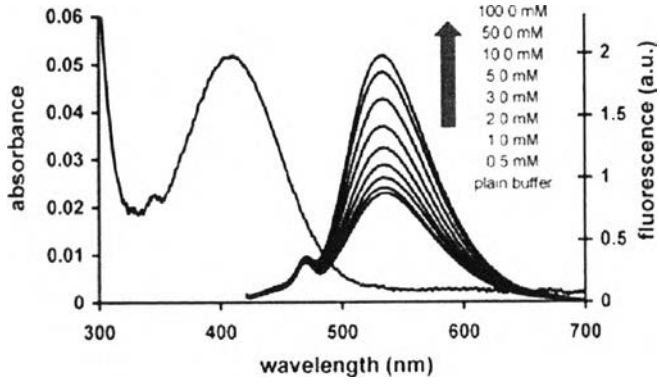

Figure 2.7 Absorbance spectra of sensor **3** at λ_{max}= 410 nm, and fluorescence spectra of compound **3** with different amounts of fructose.

Table 2.3 The association constants (K_a) of sensor **3**

Sugar	K_a/M^{-1}
D-Fructose	170
D-Galactose	8
D-Glucose	2
Ethylene glycol	0.5

Wang *et al.* [83] designed a new saccharide sensor based on a tetrathiafulvalene-anthracence dyad with a boronic acid group as shown in Fig. 2.9 a). They reported that the fluorescence spectra of dyad **4** displayed the fluorescent enhancement in the presence of different concentration of fructose as shown in Fig. 2.9 b). This phenomenon can be explained by photoinduced electron transfer (PET) between the excited anthracene and TTF units. The fluorescence quenching of dyad **4** is caused by boronic acid group, which is a weak electron acceptor, while the TTF unit displays strong electron donating. Thus dyad **4** shows weak fluorescence. After addition of fructose, the dyad **4** did not allow the effective PET process.

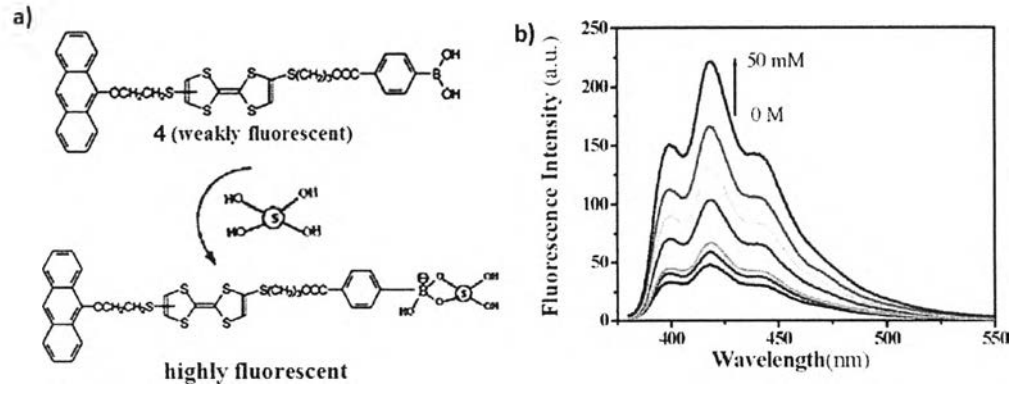

Figure 2.8 a) proposed interaction of dyad **4** with saccharide in aqueous solution b) fluorescence spectra of dyad **4** with different concentration of fructose in THF/H_2O (1:1, v/v) with 0.033 M phosphate buffer at pH 7.3

Under identical conditions, the fluorescence intensity of dyad **4** with other saccharises such as glucose, galactose and mannose as shown in Fig. 2.10. The fluorescence of dyad **4** with glucose or galactose or mannose exhibited a small fluorescence enhancement. Compared to fructose, the degree of the fluorescence enhancement for glucose, galactose and mannose is relatively small.

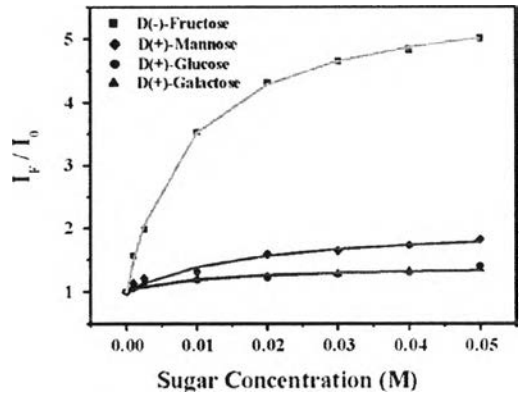

Figure 2.9 Fluorescence intensity of dyad **4** with different concentration of saccharide (fructose, glucose, galactose and mannose) in THF/H_2O (1:1, v/v) with 0.033 M phosphate buffer at pH 7.3

For the data shown in table 2.4, the association constants of dyad **4** with other saccahrides suggest that dyad **4** binds strongly with fructose over mannose, glucose and galactose.

Table 2.4 The association constants (K_a) and fluorescence intensity changes (I/I_0) of dyad **4** with different saccharides

sugar	K_a	I/I_0
fructose	115 ± 2 ($r^2 = 0.99$)	5.01
mannose	81 ± 8 ($r^2 = 0.98$)	1.82
glucose	70 ± 7 ($r^2 = 0.98$)	1.40
galactose	66 ± 7 ($r^2 = 0.98$)	1.35

Zhang *et al.* [84] have created dyad **5** based on nitroyl nitroxide-anthracene for sensing saccharides. The dyad **5** showed characteristic emission band at 419 nm. As shown in fig 2.11(a), the fluorescence spectra of dyad **5** with fructose displayed fluorescence enhancement at 419 nm due to the competitive binding of saccharides with compound **7**. These results can be described that the binding constant of boronic acid group of compound **6** with fructose was larger than that of the boronic acid group of compound **6** with two phenolic –OH groups of compound **7**.

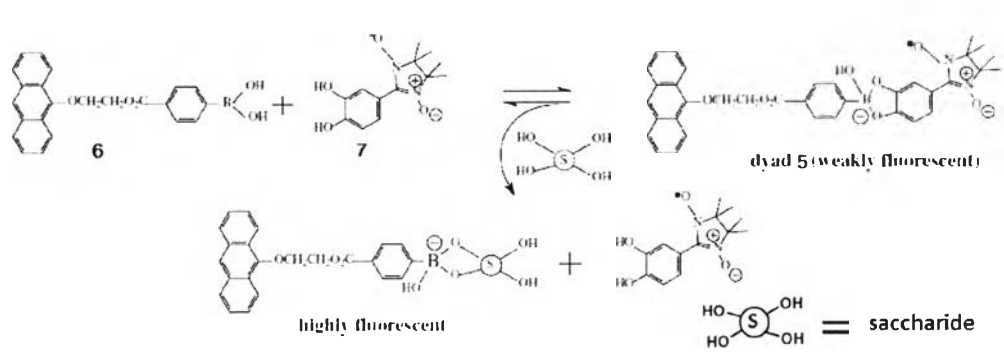

Figure 2.10 Proposed structures of compounds **6** and **7** and the competitive binding of saccharides with compound 6 in the presence of compound **7**

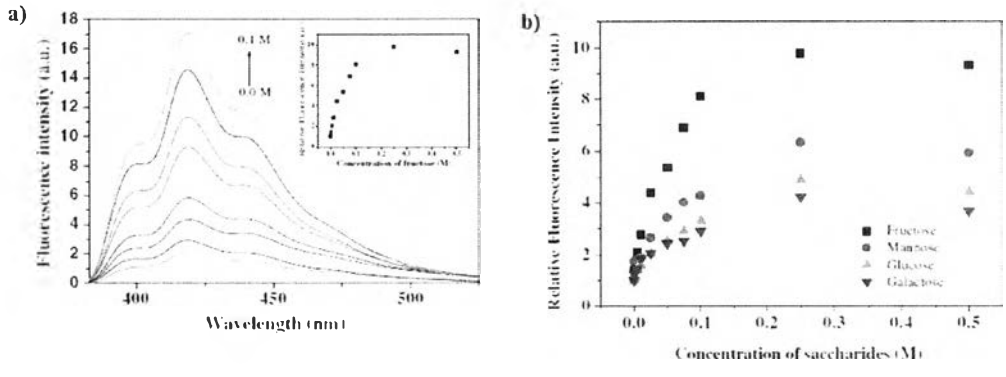

Figure 2.11 a) fluorescence intensity of dyad **5** with different amounts of fructose in THF/H_2O (1:1, v/v) with 0.033 M phosphate buffer at pH 7.3 and excitation wavelength 370 nm; the inset shows the relative fluorescence intensity at 419 nm versus the fructose concentration. b) the relative fluorescence intensity at 419 nm of dyad **5** with different concentration of saccharides (fructose, glucose, galactose and mannose) in THF/H_2O (1:1, v/v) with 0.033 M phosphate buffer at pH 7.3

Moreover, the fluorescence intensity of dyad **5** versus other saccharises such as fructose, glucose, galactose and mannose led to fluorescence enhancement. Figure 2.12(b) displayed the largest fluorescence enhancement for fructose in THF/H_2O (1:1, v/v) with 0.033 M phosphate buffer at pH 7.3. This result corresponds to the binding constants of boronic acid and fructose.

2.1.2 Determination of saccharides using the modified AuNPs

Manju and Sreenivasan [85] designed **RBITC@AuNPs** for saccharides prepared by the generation of dextran coated AuNPs (**Dex@AuNPs**) and further conjugation of aminophenyl boronic acid (**ABA**) to **Dex@AuNPs** by Schiff base formation to obtain the aminophenyl boronic acid functionalized AuNPs (**ABA@AuNPs**). Finally, the rhodamine B isothiocyanate (**RBITC**) was adsorbed onto **ABA@AuNPs** by hydrogen bonding to obtain **RBITC@AuNPs**. The fluorescence spectra of **RBITC** showed a strong fluorescence while the fluorescence of **RBITC** adsorbed on AuNPs (**RBITC@AuNPs**) exhibited a weak fluorescence as shown in Fig. 2.13.

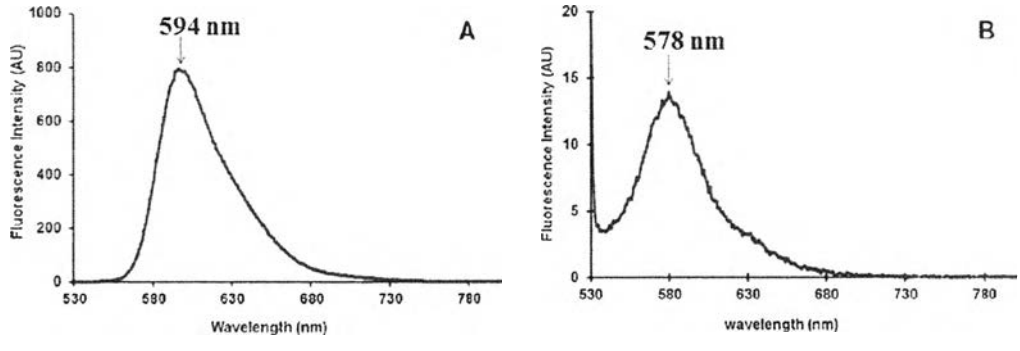

Figure 2.12 Fluorescence spectra of a) **RBITC** b) **RBITC@AuNPs** in aqueous solution of synthetic tear fluid

It is well known that AuNPs as excellent quenchers by energy transfer mechanism [86] and a minimum distance between AuNPs and the fluorophore leads the effective fluorescence quenching [87]. Fluorescence peak of **RBITC** exhibited blue shifted from 594 nm to 578 nm due to more hydrophobic environment by the fluorophore (**RBITC**) adsorbed on AuNPs close to the aromatic moiety of aminophenyl boronic acid [88].

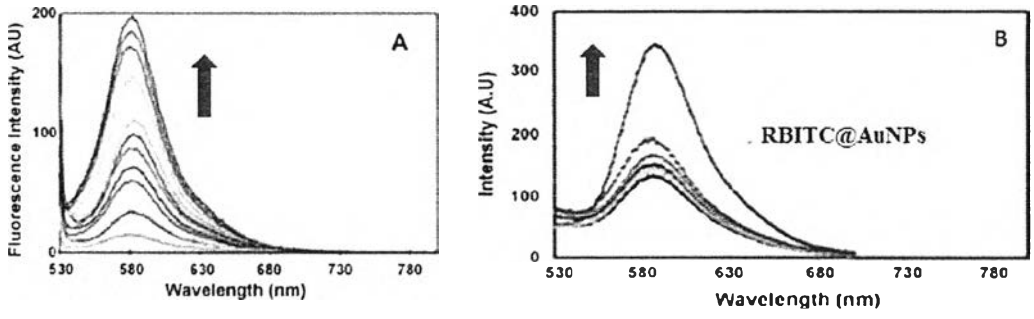

Figure 2.13 Fluorescence intensity of a) **RBITC@AuNPs** in the presence of glucose b) **RBITC@AuNPs** in the presence of glucose in real tear fluids

The fluorescence spectra of **RBITC@AuNPs** upon the increment of glucose displayed fluorescence enhancement as shown in Fig 2.14 a). This phenomenon can be described in the attribution of the isolation of fluorophore (**RBITC**) from **ABA@AuNPs** by glucose binding and the aggregation of the particles produces a larger size of gold nanoparticles while the distance between **RBITC** and AuNPs was increased by glucose swelling.

2.2 Objective and scope of this research

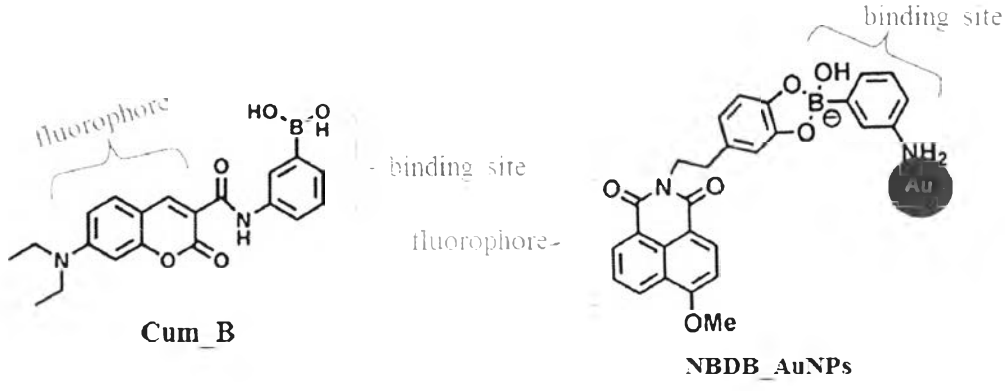

Figure 2.14 Structure of fluorescence sensor **Cum_B** and **NBDB_AuNPs**

The aim of this research is to develop the fluorescence sensors for determination of saccharides. We intend to utilize boronic acid for recognition in aqueous sensing application. Therefore, two fluorescence sensor **Cum_B** and **NBDB_AuNPs** were designed and synthesized in the research. As displayed in Fig 2.15, the sensor **Cum_B** consists coumarin unit as fluorophore and boronic acid as a binding unit to covalent bound toward saccharides. Additionally, we attempt to apply fluorescence sensory molecules to incorporate in the nanoparticles with the expectation of improvement of fluorescence response for detection. Moreover, the novel sensor **NBDB_AuNPs** contains napthalimide as fluorophore moiety and boronic acid as a binding site and then they are self-assembly on AuNPs to yield **NBDB_AuNPs** as fluorescence probe.