Macrocycle encapsulation triggered supramolecular pK_a shift: a fluorescence indicator for detecting octreotide in aqueous solution

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ABSTRACT: Supramolecular pK_a shifts, have attracted much attention in catalytic and biomimetic studies because of their excellent property to modify the acidity or basicity of the substrate in aqueous media by host-guest inclusion. Here, Fluorescence indicator displacement based on cucurbit[8]uril encapsulation of the dye acridine leads to the recognition of the peptide drug octreotide in aqueous solution via distinctive pH signals was expolited. This is thought to be a result of competitive host-guest interactions involving a supramolecular pK_a shift.

KEYWORDS: Supramolecular pK_a shift; Cucurbit[8]uril; Fluorescence indicator displacement; Drug octreotide; Acridine

1. Introduction

Fluorescent chemosensors have been widely applied for ion detection in fields as diverse as biology, pharmacology, physiology, and the environmental sciences. This stems from their high sensitivity/detection limits, ease of use and ability to provide results quickly [1-9]. However, several problems and challenges still exist with regard to the efficient recognition of neutral analytes, such as amino acids or peptides, primarily because such molecules lack suitable complementary binding sites with fluorescent chemosensors, especially in aqueous solution.

To address this issue, the host–guest binding triggered fluorescent dye properties of macrocyclic hosts have been explored as a possible alternative to traditional

chemosensors [10, 11]. In this approach, the host molecule can create a masking environment around the fluorophore guest via the use of non-covalent interactions, which allows for the low cost and efficient modification of the physicochemical properties of the dye molecules. These can be further termed as host/dye fluorescence indicator displacement (FID) systems, and have been reported previously for macrocycles [12-16], which are able to recognize and detect many important biologically and environmentally relevant species. Cucurbit[*n*]urils (Q[*n*]s or CB[*n*]s) [17,18], are a relatively new class of macrocyclic receptor and are showing promise as building blocks in supramolecular chemistry. In particular, the rigid hydrophobic cavity and two identical carbonyl fringed portals available to Q[*n*]s enable them to display some unique affinities and photophysical properties when employed as fluorescent/absorbing dyes in aqueous media [19-22]. However, the application of Q[*n*]/dye as an FID sensing system is far less exploited [23-28]. In particular, scant attention has been paid to the encapsulation ability of a macrocyclic host and subsequent triggered pK_a shifts of dye molecules. It is noteworthy that supramolecular pK_a shifts [29-32], induced by macrocyclic host complexation, have proved to be a simple and efficient way to modify the acidity or basicity of a substrate in solution, and this paves the way for the application of host–guest complexes in catalytic and biomimetic studies.

Acridine dye (Ac), an *N*-heterocyclic aromatic compound, has attracted increased interest given its unusual chemical and photophysical properties [33]. Generally, in aqueous solution acridine exists in two prototropic forms (pK_a ~5.3),

namely the neutral (Ac) and protonated (AcH^+) forms. It exhibits characteristic fluorescence spectra for both of these forms, which allows acridine derivatives to be used as pH probes [34]. This has led to related applications in the investigations of micro-heterogeneous and confined media such as micelles [35], cyclodextrins and calixarenes [36]. For example, Mohanty and co-workers revealed that the protonated form of Ac exhibits a stronger interaction with *p*-sulfonatocalix[6]arene than does the neutral form [37], which leads to a large upward pK_a shift (~2 units) for the dye. More recently, the same group discovered that the strong affinity of Q[7] and Q[8] for the protonated form of Ac resulted in a large upward pK_a shift ($\Delta pKa \sim 3.4$ units for Q[7] and ~1.3 units for Q[8]) in the dye, which is associated with strong binding ability [38]. Herein, we have utilized the host-guest interaction of Q[8] with the 'Ac' dye together with the dyes photophysical properties as an FID sensing system to detect the peptide drug octreotide (Oct) under different pH environments in aqueous solution (Fig. 1). To our knowledge, this is the first study that exploits the significant pk_a shift of the acridine dye (Ac/AcH+) in the presence and absence of a macrocyclic host and utilizes the resulting fluorescence response signal.

2. Materials and methods

2.1 Instruments

UV-vis absorption and fluorescence emission spectra of the host-guest complexes were recorded on an Agilent 8453 spectrophotometer and Varian RF-540 fluorescence spectrophotometer, ^1H NMR spectra were determined at 400 MHz with a Nippon Denshi JEOL FT-400 spectrometer with Me4Si as an internal reference.

2.2. Materials

In the study, peptide drug octreotide and acridine dye (Ac) were obtained from Aldrich. Perchlorate salts: K^+, Na^+, Zn^{2+}, Mg^{2+}, NH_4^+, Ca^{2+}; tetrabutylammonium salts: Cl^-, $C_2O_4^{2-}$, SO_4^{2-}, $H_2PO_4^-$; organic sample: uric acid, urea, hydroxyproline, and glucose, were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Cucurbit[8]uril (Q[8]) [17,18] were prepared and purified according to the literature and developed method in our laboratory.

4.3. Methods

For UV-vis absorption and fluorescence emission studies of Ac in the presence of Q[8] host, aqueous solutions of Ac were prepared with a fixed concentration of 1.0×10^{-5} M at different pH values, and the samples of these solutions were combined with Q[8] to give solutions with a ratios ranging between of 0 and 2.0 equiv. For UV-vis absorption and fluorescence emission studies of Q[8]/Ac in the presence of octreotide, aqueous solutions of Q[8]/Ac (2:1, mole ratio) were prepared with a fixed concentration of 1.0×10^{-5} M of Ac at different pH values, and the samples of these solutions were combined with octreotide to give solutions with a ratios ranging between of 0 and 2.0 equiv.

3. Results and discussion

Considering the ground state pK_a value of the acridine dye is about 5.3 [39], (see prototropic equilibria of the AcH^+ and Ac forms in aqueous solution Table S1), when the vast majority exists as the protonated AcH^+ form of the dye, the solution should be acidic (pH<4, >95% abundance) and displays green fluorescence emission (480 nm). By contrast, when the majority is the neutral Ac form, the solution should be alkaline

(pH>7, >98% abundance) and the system exhibits a blue fluorescence emission (432 nm). Meanwhile, it is possible that both species AcH^+ and Ac could co-exist in aqueous media in the case of 4<pH<7, and the related fluorescence emission should be located between the green and blue colour range according to the additive colour theory (460 nm). As a result, three distinct fluorescent signals can be triggered by the Ac dye at different pH values. Taking into account that the pK_a value of the Ac dye changes from 5.3 to 6.6 upon complexation by the Q[8] host [38], most of the AcH^+ species can survive in acidic solution below pH 5.3 by accommodating itself within the Q[8] cavity (Table S2). In other words, as shown in Fig. 2, the different host-guest interactions of Q[8] with AcH^+ or Ac as FID sensing species and the resultant fluorescence signal can be selectively assembled and tuned by judicious control of the pH value due to the novel pK_a shift of the Ac dye.

As described in Fig. 2, there are three host-guest complexation modes for Q[8] and the dye according to the host encapsulation triggered prototropic equilibria of the AcH^+ and Ac forms in aqueous solution, namely Q[8]·$(AcH^+)_2$ (pH<5.0), Q[8]·$(AcH^+)_2$, Q[8]·[(AcH^+) (Ac)] and Q[8]·$(Ac)_2$ (5.0<pH<8.0), and Q[8]·$(Ac)_2$ (pH>8.0). Therefore, the detailed host-guest binding information of Q[8] with the Ac dye at different pH values was first evaluated. As shown in Fig. S1, the protonated AcH^+, Ac and even the mixture of both forms of the dye display the same strong and sharp absorption peak at ~354 nm and another broad and weaker absorption band centered at ~400 nm along with a shoulder at ~340 nm. On the addition of Q[8]·to an aqueous solution of acridine in different pH conditions. The UV-vis spectra suggest

that all the different Ac species undergoes similar changes, and the absorbance of at 354 nm decreased significantly with a slight hypsochromic shift of ~2 nm. No obvious spectral changes were observed for the other absorption peaks.

Interestingly, the fluorescence spectra of the free Ac species exhibited the typical characteristic emission peaks around 480 nm (AcH^+), 460 nm (mixture of AcH^+ and Ac), and 432 nm (Ac) at pH 2.0, 4.5, 7.0, respectively (Fig. 3a-c). Upon addition of Q[8]·to the solution, as show in Fig. 3a-c, the fluorescence intensity monitored at 480 nm, 460 nm, and 432 nm displayed a dramatic decrease respectively, without any change in their maximum emission peaks. This result suggests a similar interaction between the Q[8] host and the different Ac forms under different pH conditions. A stoichiometric analysis by the Jobs plot method suggested that the host-guest complex of Q[8] with the guests possessed a 1:2 stoichiometry (Fig. S2). Consequently, the fluorescence emission quenching in the host-guest complexes can be ascribed to the formation of a H-dimer of AcH^+ or Ac in the rigid cavity of the Q[8] host. The binding constants (K_a) were determined to be 1.81×10^5 M^{-1} and 9.00×10^4 M^{-1} for Q[8]·$(AcH^+)_2$ at pH 2.0 and 4.5, respectively, whereas K_a for the host-guest complex at pH 7.0 was found to be 5.2×10^4 M^{-1} (Fig. S3). The much weaker binding constant here compared to the species in acidic solution, may be is attributed to the co-existing of Q[8]·$(AcH^+)_2$ and Q[8]·$(Ac)_2$ in neutral solution due to the pK_a shift (Fig. 2). The protonated form of the dye has a higher binding constant with the Q[8] host given the strong ion-dipole interactions between the cationic AcH^+ guest and the negative carbonyl fringed portals of the host. The stoichiometry and K_a of the Q[8]/dye system

were further confirmed from isothermal titration calorimetry (ITC) experiments (Fig. S4). However, because of the solubility limitations associated with Q[8] and Ac·in neutral aqueous solution, titration of Ac with Q[8] in neutral and alkaline conditions could not be carried out, and the binding constant for the neutral form with Q[8] could not be estimated reliably.

Octreotide (Oct), as a therapeutic peptide [40], is a synthetic analogue of somatostatin, and presents an *N*-terminal phenylalanine and tryptophan, which is known to act as a guest for the Q[*n*] host [41]. Oct reduces circulating levels of both growth hormone and insulin-like growth factor I. Additionally, it is indicated in acromegaly, the symptomatic treatment of carcinoid and vasoactive intestinal peptide tumors, and hyperinsulinism in children who are not responsive to Diazoxide [42, 43]. Since Oct is excreted intact in the urine [44], it thus offers a simple and efficient measure of the circulating concentration after dosing and is very important in clinical treatment. ITC results suggested that the K_a of the host-guest interaction of Q[8] with Oct is 1.30×10^6 M^{-1} and 8.28×10^5 M^{-1} with a stoichiometry of 1:2 in acidic solution and neutral solution, respectively (Fig. S5). Therefore, the system of Q[8] with AcH^+ or Ac species as an FID assay for the detection of Oct was essentially can be assessed.

As show in Fig. 3d-f, nearly all of the quenched fluorescence emissions were recovered on increasing the concentration of the sample of Oct added to the solution of the FID system under different pH conditions. This is because the Oct acts as a competitive binder. Meanwhile, the respective UV-vis spectroscopic data at 354 nm were also recovered (Fig. S6). Consequently, there is no doubt that the fluorescence

was recovered and can be ascribed to the dyes release from the Q[8] cavity. Interestingly, closer inspection suggested that the competitive binding of Oct to the Q[8] cavity actives the fluorescence signal and different features can be attributed to the novel host-guest interaction induced pK_a shift of the dye. For example, as the pH of the medium reaches 2.0, which is lower than the pK_a value (5.3) of the dye and the Q[8]/dye complex (pK_a 6.6), the minority uncomplexed AcH^+ remains in solution and an emission maxima at ~480 nm is observed. Following addition of the Oct sample to the solution, the fluorescence emission maxima at ~480 nm was retrieved without any other peak changes because the released dye remains in the protonated form at this pH. However, as the pH of the medium reaches 4.5, which is close to the pK_a value (5.3) of the dye, but is still lower than the pK_a value (6.6) of the Q[8]/dye complex, Q[8]·$(AcH^+)_2$ is the sole species in solution, whereas when AcH^+ was released from the Q[8] cavity by the competitive binder Oct, some of the AcH^+ is converted to the Ac form according to the prototropic equilibria of the AcH^+ and Ac forms at pH 4.5 ([AcH^+]/[Ac], 86%-14%, Fig. 2). Interestingly, such a deprotonation procedure for AcH^+ to Ac can be readily monitored by fluorescence spectra. As shown in Fig. 3e, because of the host-guest complex equilibria of the Q[8] and the dye, the fluorescence spectra of the FID system exhibited a typical mixture emission for the AcH^+ and Ac at around 460 nm with weak intensity. After addition of the Oct to the solution, the maxima emission peak was initially retrieved at 480 nm (characteristic of the AcH^+ form released from Q[8] cavity), and then shifted to 460 nm with a broad emission spectrum (characteristic of the mixture emission of AcH^+ and Ac form in aqueous

medium) as increasing amounts of Oct were added. Notably, as the pH of the aqueous medium approached neutral (pH~7.0), the retrieved signal emission in the spectra displayed significant tunable properties from 460 nm to 432 nm (Fig. 3f). At this stage, we need to consider that the present pH value is more than the pK_a value (5.4) of the dye and the pK_a value (6.6) of the Q[8]/dye complex, and thus the emission maxima at ~432 nm should correspond to the neutral form of the uncomplexed dye. However, Q[8]·$(AcH^+)_2$, Q[8]·[(AcH^+) (Ac)], and Q[8]·$(Ac)_2$ for the Q[8]/dye complex may be co-exist as the FID system according to the complex equilibria between the Q[8] host and the dye (Table S2). Therefore, after addition of Oct to the solution, the maxima emission peak initially retrieved at 460 nm can be attributed to the mixture of AcH^+ and Ac released from the Q[8] cavity. Subsequently, all of the AcH^+ is converted into the Ac form due to the prototropic equilibria of the AcH^+ and Ac forms (Fig. 3g). The emission finally shifted from 460 nm to 432 nm, which indicated that only the neutral form of the dye remained in solution at pH 7.0.

The above results demonstrated the unique macrocycle encapsulation triggered supramolecular pK_a shift as an FID assay to detect the peptide drug Oct in aqueous solution (Fig. 4). An estimation of the interference of the selective response of the Q[8]-based FID chemosensors for Oct was conducted in the presence of commonly occurring urine species such as K^+, Na^+, Zn^{2+}, Mg^{2+}, NH_4^+, Ca^{2+}, Cl^-, $C_2O_4^{2-}$, SO_4^{2-}, $H_2PO_4^-$, uric acid, urea, hydroxyproline, and glucose at different pH conditions (Fig. S7). The fluorescence intensity was almost identical to that obtained in the absence of other interfering ions, indicating that the Q[8]/dye systems can be used as efficient

selective chemosensors for Oct. The detection limits were determined to be 2.81×10^{-7} M, 4.31×10^{-7} M, and 2.43×10^{7} M for Oct at pH 2.0, 4.5 and 7.0, respectively (Fig. S8).

In order to obtain more detailed information and evidence for the encapsulation and selectivity for the targeted analytes, the competitive binding between the dye and Oct molecules in the Q[8] cavity was evaluated by ^{1}HNMR spectroscopic experiments. As shown in Fig. 5, upon addition of 0.5 equiv. of Q[8] host to the acidic aqueous solution of Ac (pD 2.0), remarkable resonance peak changes corresponding to the protons of the AcH^{+} were observed. Clearly, all the protons on the dye undergo an up-field shift indicating that the AcH^{+} dyes were fully included in the Q[8] cavity. On further addition of Oct to the solution, it was noted that nearly all of the protons of the AcH^{+} shifted back to their original position, and some of the aromatic protons on the Oct molecules were shifted upfield. As a control experiment, the host-guest interaction of Q[8] with Oct in the absence of AcH^{+} was carried out. The similar upfield proton shift of Oct suggested that the aromatic group (which may be on both of the tryptophan and phenylalanine moieties due to the complicated molecular structure of the peptide) [45] of Oct, was deeply encapsulated into the Q[8] cavity and thereby caused the AcH^{+} to be released from the Q[8] cavity via a competitive binding process.

4. Conclusions

In summary, we have developed a new type of supramolecular pK_a shift triggered turn-on fluorescent chemosensor using macrocyclic-based host-guest interactions as

an FID system for the detection of a peptide drug in aqueous solution. This study demonstrates for the first time that the host-guest interaction switched pK_a shift is useful for target analysis under different pH environments with multiple fluorescent signals. We thus believe that the present work will provide a new direction for the role of supramolecular pK_a shifts in molecular sensing, detection, and recognition.

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Figure Captions

Fig. 1. (a) Chemical structure and representation of Q[8], AcH^+, Ac, and Oct; (b) illustration of the plausible FID process based on host-guest interactions with distinct pH response signals for Oct in aqueous medium.

Fig. 2. Illustration of the pK_a of the dye in the absence and presence of the Q[8] host.

Fig. 3. Fluorescence spectra changes of Ac dye (10 μm) in the presence of increasing concentrations of Q[8] (20 μm) at pH 2.0 (a), 4.5 (b), and 7.0 (c), respectively, and the thereby fluorescence spectra changes of Q[8]/Ac in the presence of increasing concentrations of Oct (20 μm) at pH 2.0 (d), 4.5 (e), and 7.0 (f) with an excitation at 350 nm, respectively; (g) the plausible mechanism of Q[8]-based FID fluorescence signal changes for Oct. λ_{ex} = 350 nm.

Fig. 4. Fluorescence spectra of the Q[8]/dye (a) and relative fluorescence intensity

changes (b) in aqueous solution upon addition of various ions and organic species (20 μM) as their aqueous solution at pH 2.0, 4.5, and 7.0, respectively. λ_{ex} = 350 nm.

Fig. 5. ^{1}H NMR spectra of Ac (1.0 mM, 400 MHz, D_2O, pD 2.0) (a) in the presence of Q[8] (0.5 mM) (b), (c) the addition of Oct (1.0 mM) to the solution of b, and the ^{1}H NMR spectra of Oct (1.0 mM, 400 MHz, D_2O, pD 2.0) in the presence (d) and absence of Q[8] host (e). The Q[8] protons are labeled as (●).

Fig.1.

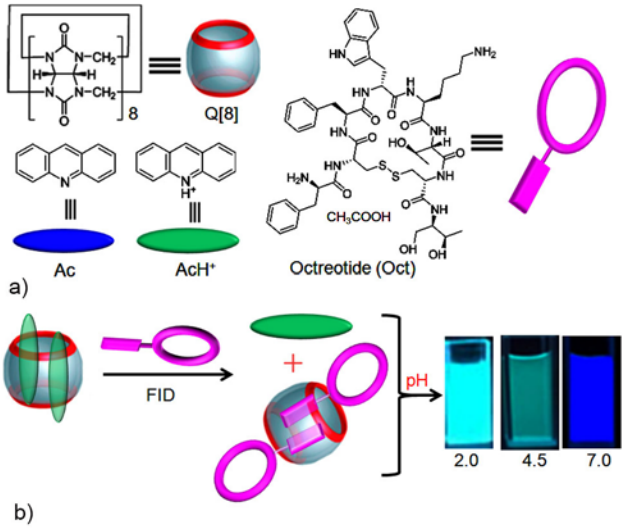

Q[8]
Ac
AcH+
Octreotide (Oct)
a)
FID
pH
2.0
4.5
7.0
b)

Fig.2.

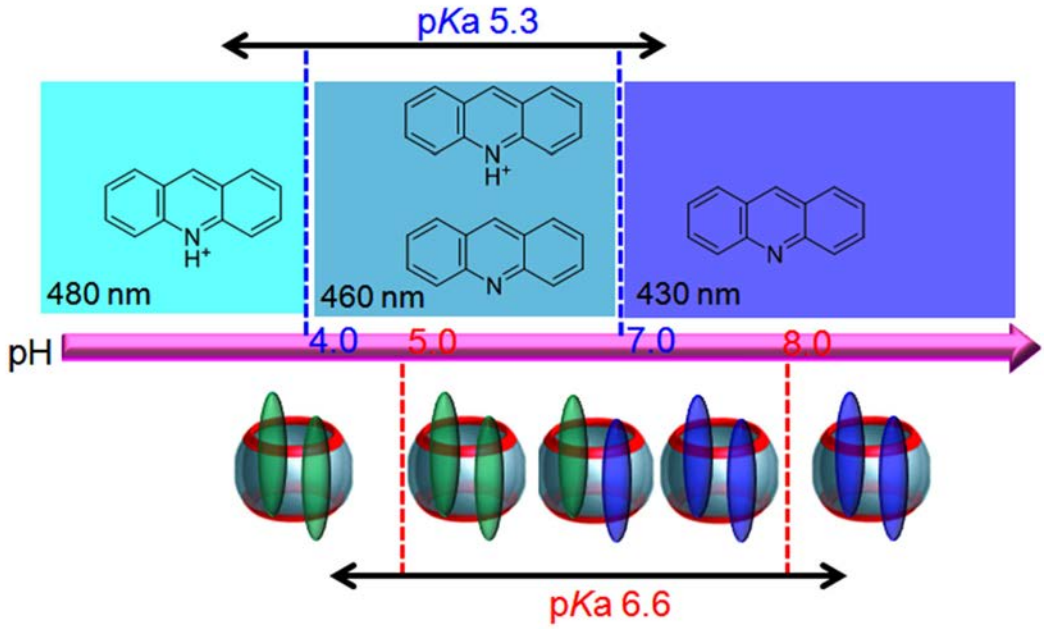

pKa 5.3
480 nm
460 nm
430 nm
pH
4.0
5.0
7.0
8.0
pKa 6.6

Fig.3.

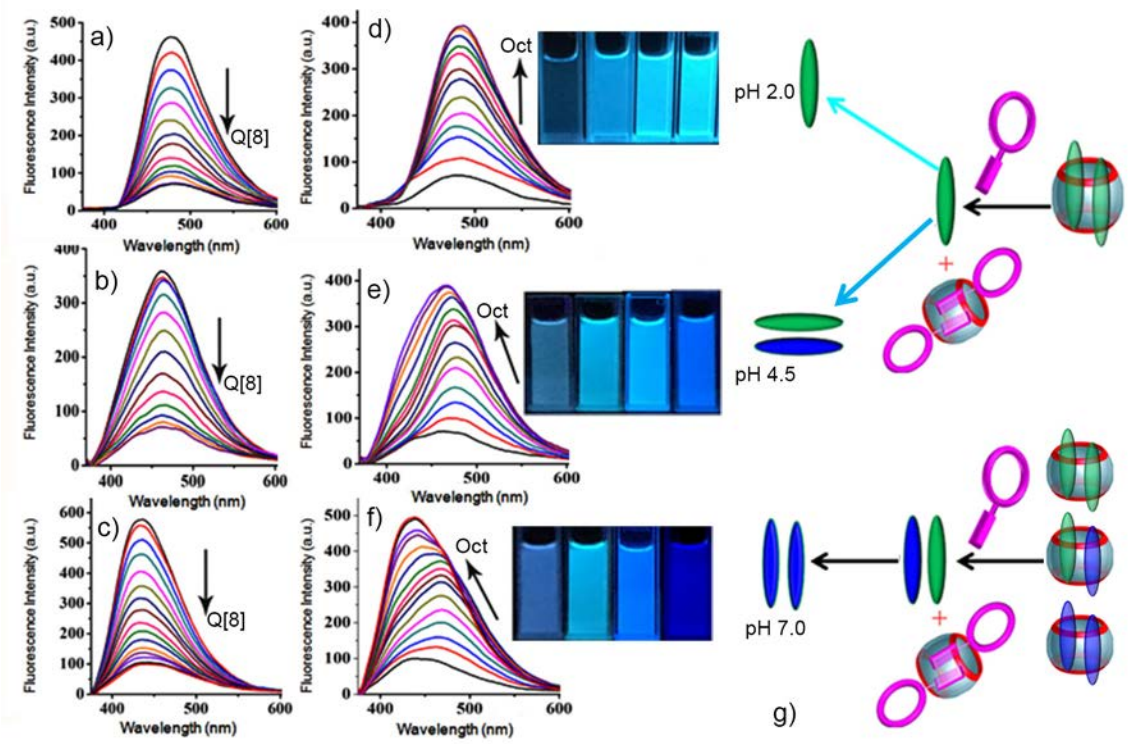

a)
b)
c)
d)
e)
f)
g)
Q[8]
Oct
Fluorescence Intensity (a.u.)
Wavelength (nm)
pH 2.0
pH 4.5
pH 7.0

Fig.4.

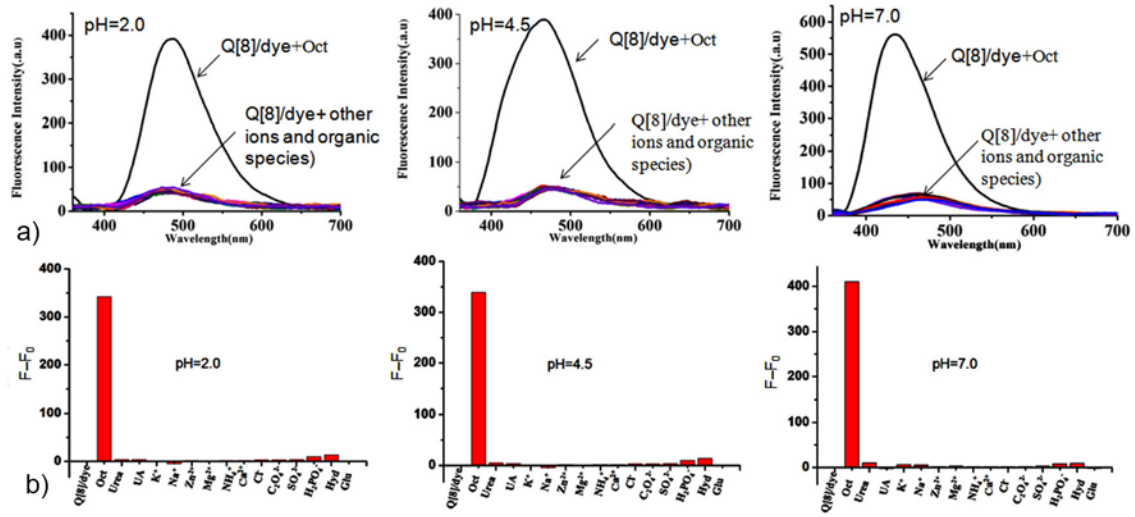
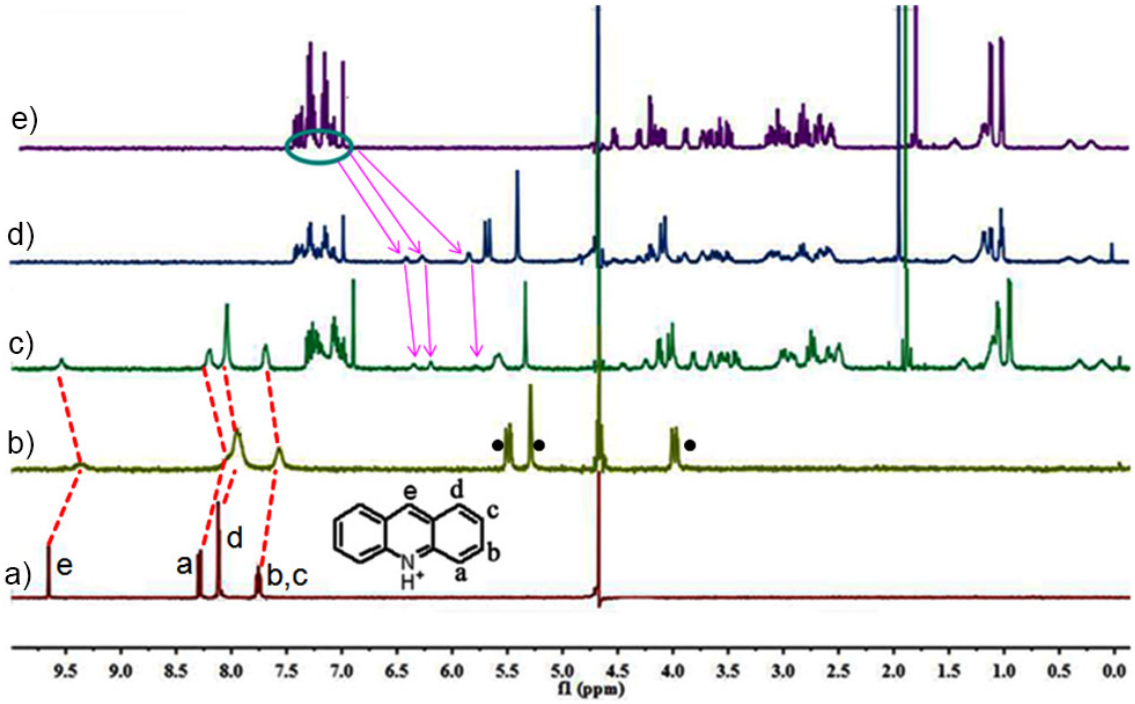

pH=2.0
pH=4.5
pH=7.0
Q[8]/dye+Oct
Q[8]/dye+ other ions and organic species)
Fluorescence Intensity(a.u)
Wavelength(nm)
a)
b)
F–F0
Q[8]/dye
Oct
Urea
UA
K+
Na+
Zn2+
Mg2+
NH4+
Ca2+
Cl-
C2O42-
SO42-
H2PO4-
Hyd
Glu

Fig. 5.


e)
d)
c)
b)
a)
e
a
d
b,c
e
d
c
b
a
N
H+
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)