Chemical Sensing of Ions and Its Clinical Applications

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Abstract : The basics created in the past years have led to substantial applicability and the time has reached to develop the commercial product for the society. However, continuous fundamental research is needed for better and alternative types of several sensors. Specially, more research is needed to improve the methods for selective molecular recognition as well as sensing (in particular of the clinically significant organic analytes; from saccharides to creatinine) in physiological conditions. Other types of sensors are still needed that have not been realized so far.

Key word : Fluorescent signaling, Clinical Applications, Ions, Chemical Sensing.

Introduction

Chemists define sensors as “a receptor that interacts with an analyte producing a detectable change in a signal” (Balzani and Scandola, 1991). Chemical sensing of ions using fluorophore as a signaling unit has shown wide interest among chemists and biologists because molecular fluorescent signaling is the natural interface between human and molecular domains via the intermediacy of the photon. It has numerous advantages over various other sensing techniques which can be summarized as high sensitivity of detection down to the single molecule, on-off switchability, and can serve as analytical information (Valeur, 2002). The largest single group of chemical sensors comprises, at present, sensors based on the use of fluorescent probes or labels. The growing need for continuous monitoring in various areas including bioprocess control, critical care medicine, in environmental sciences, the nuclear industry, marine sciences and numerous other areas has certainly increased exponentially in the field of optical sensing (Prasanna de Silva *et al.*, 1997). Specially, development of chemical sensors has a great future in clinical applications (Czarnik, 1994). For example, analysis of quickly-changing blood analytes is crucial to adequately evaluate, stabilize and manage critical care patients. Therefore, the development of practical and inexpensive sensors and systems for the clinical determination of critical care analytes like blood gases (O_2, CO_2, pH), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-), and certain metabolites (glucose, lactate, urea, creatinine) in whole blood remains an important area of research (Bryan, 1989). It is important to have immobilized sensors for these applications mentioned. In a typical case, a fluorescent indicator is immobilized on a polymeric support and this "smart" material responds to the presence of an analyte by a change in its fluorescence. This article summarizes the development of PET based chemical sensors for metal ions and a representative examples for in vitro diagnostic of PET based sensing systems measuring critical care analytes in whole blood.

Photoinduced Electron Transfer (PET) Based Chemosensors.

Fluorescent signaling via the PET strategy is distinguished by its intrinsically supramolecular nature since distinct components perform each one (or more) of the necessary functions (Fig. 1). Basic components require for designing a PET based chemosensors are Flurophore **(F)**, Spacer **(S)** and a Receptor **(R)** (Valeur and Leray, 2000). A fluorophore module is the site of both photonic transactions of excitation and emission. A receptor module is responsible for guest complexation and decomplexation. A spacer module holds the fluorophore and receptor close to, but separate from, each other. This also means that true molecular engineering applies, *i.e.*, the optical, guest-binding, and redox properties of the components allow the quantitative prediction of the signaling parameters of the supramolecular system. Further, PET signaling systems have natural "all or none" switchability: guest-induced "off-on" and "on off" fluorescence are both designable.

The pioneering work of Weller about 40 years ago (Weller, 1968), provides the thermodynamic basis of PET. Figure 2 provides a summary in terms of frontier orbital energies. It also shows how PET system employ thermal back-electron transfer as a self-repair mechanism following the potentially damaging PET process. Upon excitation of the fluorophore, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), fluorescence in this molecule is observed when this excited electron in LUMO goes to the HOMO releasing the excess of energy as light. It might also happen that a completely filled orbital from another part of the molecule or from another molecular entity could have energy between that of the HOMO and LUMO of the fluorophore. When this orbital is full (donor group), a photoinduced electron transfer from this filled orbital to the HOMO of the fluorophore can take place. A further electron transfer from LUMO of the fluorophore to the external orbital retrieves the ground state. These process results in the quenching of emission intensity or other words no fluorescence are observed at all because the relaxation of the excited electron from the excited state to the ground state is a nonradiative decay process. A similar process can also take place when there is an empty orbital from another part of the molecule or from another molecular entity between both the LUMO and HOMO of the fluorophore. The design of chemosensors tries to take advantage of such PET effects. Since in this article we are interested in PET based chemical sensors, it

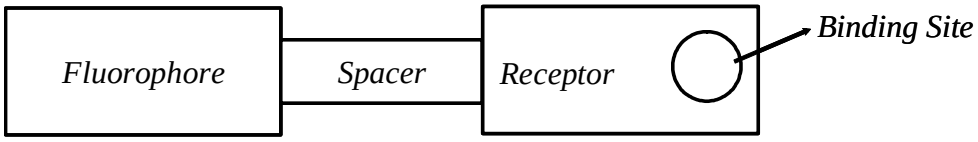


Fig. 1 : Designing format for PET-based chemosensor

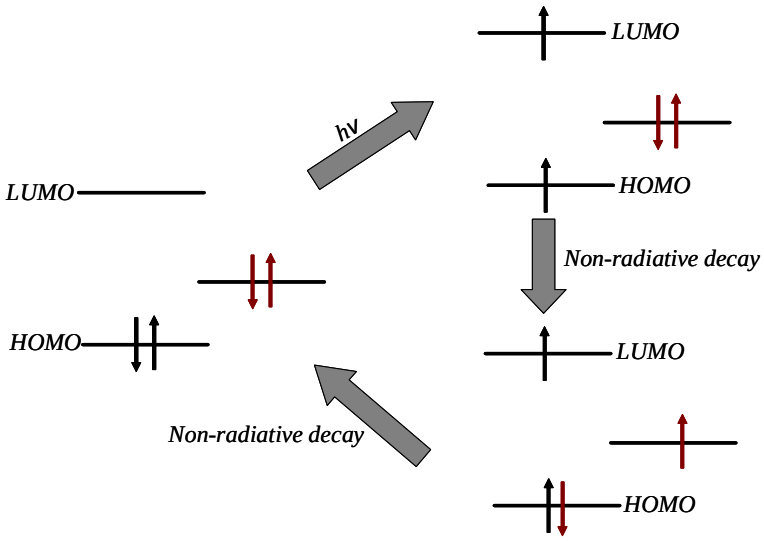


Fig. 2 : Principle of PET process with the participation of the HOMO and LUMO of the fluorophore and the occupied external orbital.

is interesting to understand the basis of the nature of the photoinduced electron transfer (PET) process that is responsible for the photophysical changes upon analyte binding. Two major events take place during the operation of a chemosensor *i.e.* "Recognition" and "Transduction". Upon analyte binding, the redox potential of the donor is raised so that the relevant HOMO becomes lower in energy than that of the fluorophore; consequently, PET is not possible any more and fluorescence quenching is suppressed. In other words, fluorescence intensity is enhanced upon analyte binding (Figure 3).

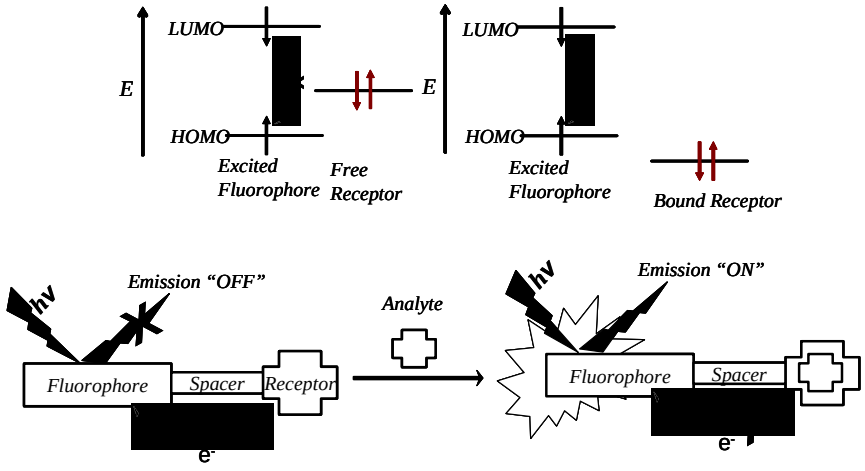
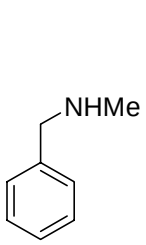


Fig. 3 : Principle of cation recognition by fluorescent PET sensors.



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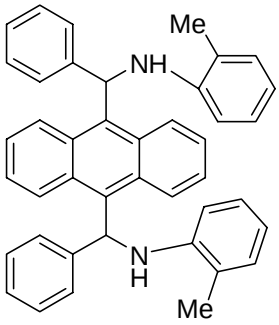

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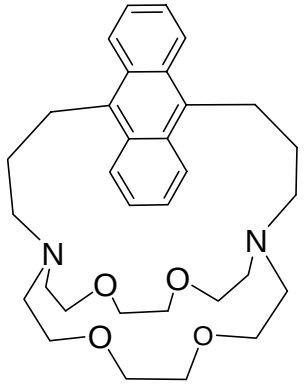
The history of the field began in the year 1976 with the compound 1 (Wang and Morrawetz, 1976), where enhancement of fluorescence due to protonation was observed: the first case of guest-induced "off-on" switching. In this case proton act as a guest and receptor is secondary nitrogen center which is separated by a methylene spacer. Nakaya's compound 2 was a near miss to be the first candidate since the low fluorescence was noted but not exploited in this aspect which was published in the year 1966 (Nakaya and Tomomoto, 1966). Of course the insights of Weller's investigations were not generally available in 1966 therefore 2 was ahead of its time.

Alkali metal ion induced fluoroionophoric system **(3)** was introduced by Lehn et al. in the late 1980's (Fages *et al.*, 1989). The cryptand 3 shows substantial fluorescence enhancements upon alkali metal ion binding. Compound 3 also binds Ag(I) because of cation-p interactions due to the anthracene lining the cryptand cavity, but no fluorescence is seen. A bifluorophoric version of 3 shows some similarities and also some interesting variations. Attachment of the



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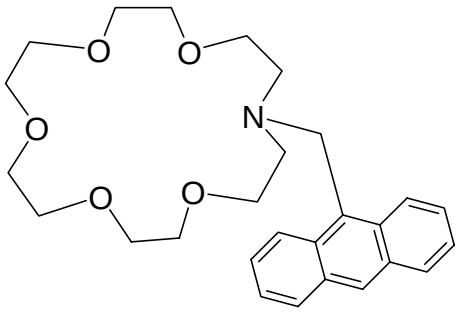

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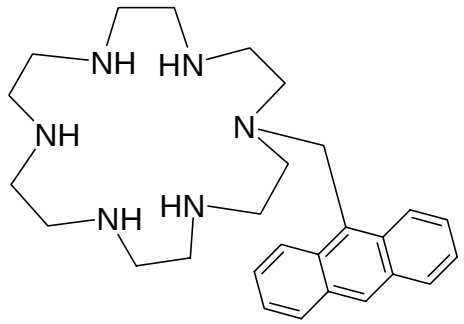

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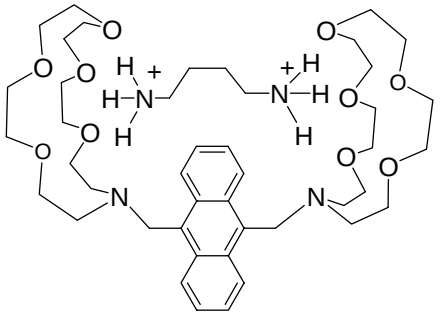

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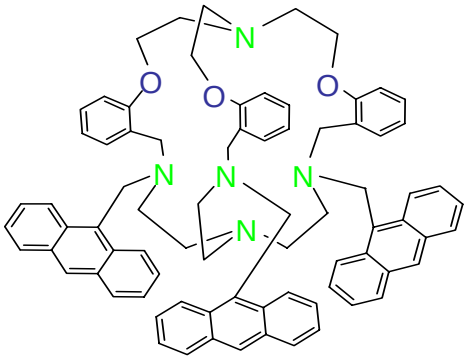

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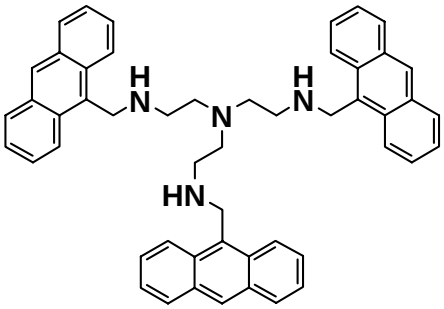

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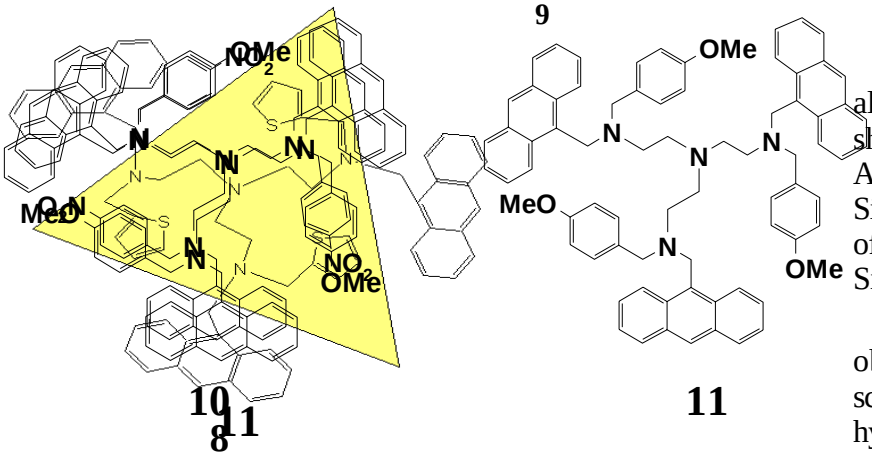

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diaza-18-crown-6 ether unit to the 1,4 positions of the anthracene reduces the intercomponent interactions compared to 3 resulting in smaller intensity effects due to alkali cations. However, significant band shifts are seen, especially with protons. Azacrown ethers like 4 developed by de Silva showed K+-switched emission in spite of its structural simplicity (de Silva and De Silva, 1986).

The presence of the basic nitrogen obviously required the use of a proton scavenger such as benzyltrimethylammonium hydroxide so that the influence of metal ions upon 4 could be unequivocally demonstrated. K^+ induced excellent 'off-on' fluorescence signalling with a fluorescence enhancement (FE) factor of 47 in methanol as solvent. Deeper investigation revealed that the binding constant between 1 and a given

alkali cation was virtually identical to the corresponding value exhibited by the parent azacrown ether.

Compound 5 containing a polyazamacrocycle was designed by Czarnik for the recognition of soft metal ions like Zn^{2+} which serve as fluorescent "off-on" switches with this metal ion given adequate pH control (Akkaya, 1986).

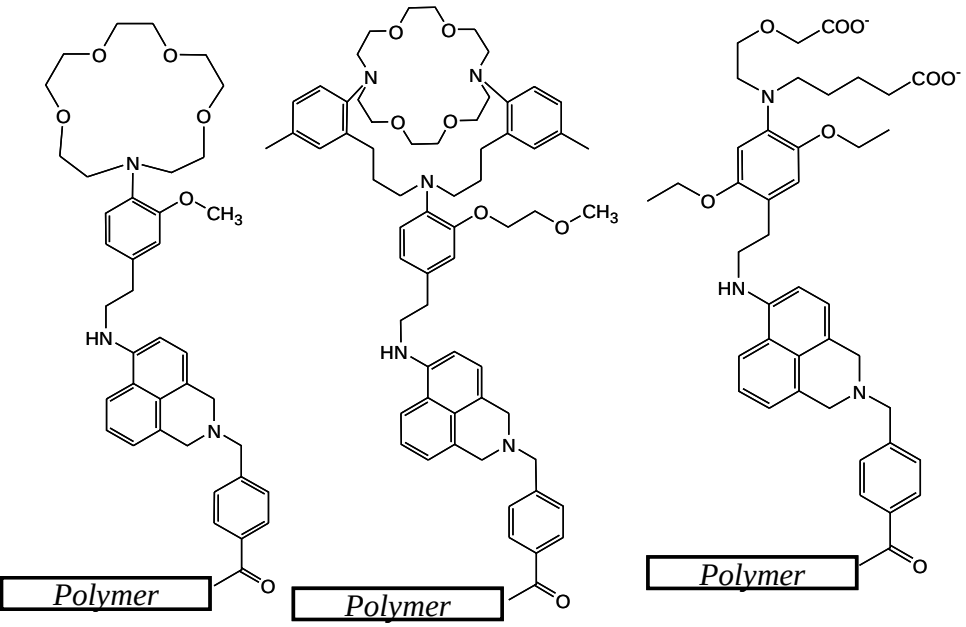
Along with the development of metal ion sensors organic diammonium systems were also started in 1990's. For example suitably positioned pairs of aza-15-crown-5 ether units 6 can signal the presence of butane-1,4-diammonium ions via an intervening anthracene fluorophore due to the complex (de Silva Sandanayake, 1990). In this case hydrogen-bonding blocks both nitrogen lone electron pairs and their possible PET activity. In 1990's a number of group worldwide became interested in this area of research (Prasanna de Silva *et al*., 1997). Till 1996, no fluorescence enhancement based chemosensor for transition metal ions were not known in the literature because transition metal ions are familiar quencher via electron transfer as well as energy transfer processes. Bharadwaj's group first reported Cu(II) and Ni(II) induced fluorescence enhancement on trianthryl heteroditopic cryptand (7) (Ghosh *et al*., 1996). In this case probably after encapsulation there is a communication gap between metal center and the signaling unit due to a layer effect by the cryptand framework. Therefore, compound 7 is distinguished by the use of d-block ions such as Cu(II) to elicit an "off on" switching response. The rigid cryptand environment around Cu(II) appears to stifle its redox activity. Cation coordination to the aliphatic amine units would then be responsible for the "off-on" switching. Ideally, OR logic gates should produce the same output when they are switched "on". Since various ions have different charge densities and coordination strengths, their abilities to enhance fluorescence will also differ. In 2002 our group introduced tripodal system (8) having N_4S_3 coordinating environment for selective sensing of Ce^{3+} in solution (Ghosh *et al.*, 2002).

Our recent designs on tripodal fluoroionophoric systems are 9, 10 and 11 have demonstrated electronic effect on PET process (Ravikumar, 2007). All three fluoroionophores show appreciably lower fluorescence compared to anthracene due to effective PET process in these systems but the quantum yield varies depending upon the nature of substitution at the PET center. In cases of 9and 11 different amount of fluorescence recovery are observed in presence of different cation inputs whereas 10 is almost inactive towards cation sensing. Detailed fluorescence emission studies on 9 and 11 in the presence of different cation inputs showed, that in, 11 having N4 donor sets bearing three p-methoxy benzyl units attached to the three nitrogen centers involving photo induced electron transfer process is a viable candidate for enhancement of fluorescence with Cu(II) input. In the absence of p-methoxy benzyl units at the nitrogen centers resulting system, 9 shows quenching of fluorescence with Cu(II).

Clinical Applications

In view of the substantial spontaneous changeability in blood gases and other quickly-changing analytes that occurs even in apparently stable patients, it has been argued

that clinical decisions should be made on the basis of trends observed with continuous, on-line monitoring (Burtis and Ashwood, 1999). However, it has become clear that, despite remarkable technological breakthroughs, truly continuous intravascular measurements are not sufficiently dependable and economic to be used routinely in critically ill patients. The next-best clinical alternative is "point of care" measurements made near the bedside, which minimize the therapeutic decision time. The needs and demand for point of care testing have continually driven the development for portable systems utilizing small disposable sensors capable of quick but accurate whole-blood measurements. Since the core of any analyzer is its sensors, one must turn to new, improved sensor technologies to meet these new requirements for small size, high stability, economy, reliability, and freedom from maintenance. He *et al*. have successfully developed polymer bound PET based systems for detection of some of the blood analytes (He *et al*., 2003). The design principle is based on photo-induced electron transfer (PET). Those types of fluoroionophores have proven highly successful as direct fluorescent cation sensing molecules, utilizing the switchable intramolecular self-quenching mechanism associated with PET. The sensing layer for each contains the immobilized fluoroionophore and is attached on top of a transparent polyester foil. The sensing layer is covered with a black hydrophilic overcoat to separate the sensing layer from optical interferents within the sample. Both the sensing and overcoat layers are hydrogels allowing the free and rapid diffusion of ions throughout the sensor, and restrict the passage of optically interfering blood constituents during the measurement time. In this aspect He *et al*. have developed three fluoroionophores 12, 13 and 14 based on



12 **13** **14**

"fluorophore-spacer-receptor" format coupled with by immobilization to hydrophilic polymers without loss of ionophore or fluorophore function. All three utilize 4-aminonaphthalimide as fluorophore, stable against hydrolysis and photo-degradation, and showing strong fluorescence characterized by the product of the extinction coefficient and the fluorescent quantum yield, and is excitable using blue LED's. In contact with aqueous solution or the aqueous phase of blood, the ionophore part of the three fluoroionophores 12, 13 and 14 reversibly bind Na^+, K^+, and Ca^{2+} respectively in neutral aqueous solutions at 37° C, pH 7.4 and 160 mmol L^{-1} ionic strength. Reversible binding of the cation to each of the ionophore moieties triggers an increase in green fluorescence from an adjacent 4-aminonaphthalimide fluorophore via PET. Unlike most ion selective measurement mechanisms, binding and signal transduction take place in a hydrophilic environment. All three fluoroionophores satisfy the system requirements for selectivity, sensitivity and spectral accessibility. These sensors are currently used in clinical analysis as "point of care" units.

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