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Trace level recognition of Zn^{2+} and Cd^{2+} by biocompatible chemosensor inside androecium, diagnosis of Pick's disease from urine and bio-mimetic β-cells exocytosis

Pritam Ghosh[1±], Koushik Pramanik[2], Suparna Paul[1,4], Pijush Malpaharia[2], Swapan Kumar Chandra[2*], Subhra Kanti Mukhopadhyay[3] and Priyabrata Banerjee[1,4**]

[1]Surface Engineering & Tribology Group, CSIR-Central Mechanical Engineering Research Institute, Mahatma Gandhi Avenue, Durgapur 713209, West Bengal, India.
[2] Department of Chemistry, Visva-Bharati University, Santiniketan 731235, India.

[3] Department of Microbiology, The University of Burdwan, Burdwan 713104, West Bengal, India

[4]Academy of Scientific & Innovative Research (AcSIR) in CSIR-CMERI, Mahatma Gandhi Avenue, Durgapur 713209, West Bengal, India.

KEYWORDS: trace level sensing of d^{10} ion, aqueous phase detection, DFT, in vitro recognition, Pick's disease diagnosis, biosensing

ABSTRACT: Two chemosensors with varying substitution have been synthesized for selective detection of d^{10} metal ion analyte Zn^{2+} and Cd^{2+} by fluorometric method from aqueous medium at very low limit of detection. Density functional theory (DFT) based *Loewdin* spin population analysis reveals that methoxy substituted chemosensor is much stronger donor than bromo substituted chemosensor. Eventually, bromo and methoxy substituted chemosensors are moderate and strong donor respectively towards selective detection of Cd^{2+} and Zn^{2+} by luminescence induced phenomenon (blue for Cd^{2+} and cyan for Zn^{2+}). The mechanism of sensing could be explained by PET-CHEF-C=N isomerisation-ILCT pathway. ^{1}H-NMR, ESI-MS and FT-IR has been carried out in order to explore the selective ion sensing mechanism. Intracellular detection of Zn^{2+} and Cd^{2+} has been carried out inside androecium (filament and pollens) of *Tecoma Stans*. Extracellular detection of Zn^{2+} for yeast cells represents the bio mimetic model experiments towards β-cells exocytosis as a marker of *diabetes mellitus*. The unprecedented and novel feature of the present biocompatible chemosensor is its application as biosensor to detect *in vivo* Zn^{2+} from human urine specimen which could be a next generation diagnostic tool for *Pick's disease*.

INTRODUCTION

In supramolecular chemistry, designed synthesis of chemosensor towards specific recognition of aqueous phase analytes have become one of the emergent areas of interest.[1-4] Among various metal ions, detection of d^{10} analyte has become particularly essential keeping in mind their indispensable application in several physiological phenomena of the human body.[5-12] However, the selective sensing of d^{10} analyte even from human body fluid has remained a great challenge. Worldwide several research groups are working in recognition of several bio benign and toxic d^{10} metal ion, however, a very few of the reported article has ever discussed regarding the designed synthesis of chemosensor towards selective recognition of analytes in aqueous phase. In view of above curiosity begins towards designed synthesis of chemosensor for selective aqueous phase identification of Zn^{2+} and Cd^{2+} at very low level.

Detection of Zn^{2+} and Cd^{2+} are necessary due to their key role in a long range of biological phenomena.[5-12] One of them is bio benign and another is heavily toxic in nature. In human brain, Zn^{2+} is connected with several endogenous proteins reversibly. Whereas, inside the cell, metallothioneins is known to bind Zn^{2+}.[13] This cation is significantt in operating a wide range of biological functions like oxidative stress response, activity of transcriptional factors, DNA repair and transcription. Significantly, over 300 enzymes inside human body require intracellular Zn^{2+} for cellular functions and pathways. In addition to several indispensable biological role, *abnormal concentration of Zn^{2+} in urine acts as an indicator in pathogenesis of Pick's disease*.[13] This is another form of dementia that causes gradual shrinking of brain cells with inappropriate personality or poor decision making of the brain. Interestingly this disease causes enhanced Zn^{2+} concentration in blood and urine. Therefore detection of Zn^{2+} from urine (normal level of Zn^{2+}: 7.6×10^{-6}M)[14] could contribute to the pathogenesis of this disease. In addition, recent report suggests that Zn^{2+} has beneficial effects in type 1 and type 2 diabetes. Zn^{2+} supplementation in diabetic patients improves the most required glycaemic control.[15-16] As a consequence it is evident that Zinc is a potential therapeutic agent for diabetes. However the deficiency of Zinc supplementation in developing countries causes current epidemic of diabetes. In particular, insulin is stored in β-cells by forming a hexamer having two Zn^{2+} ions.[17] It is released into portal venous system during de-granulation of β-cells. Therefore extracellular detection of Zn^{2+} will be helpful towards development of next generation therapeutic agent for diabetic patients as well. On the other hand, Cd^{2+} is highly lethal to human body (safe limit as per W.H.O.: 3×10^{-9}M).[18-19] Difference in ionic size leading to relative strength in Lewis acidity makes the drastic divergence between Zn^{2+} and Cd^{2+}! On one hand, Zn^{2+} is playing key role in different biological phenomena whereas on the other hand, exposure to Cd^{2+} causes serious injury to kidney, lung or nervous system. It can cause renal dysfunction or calcium metabolism in human body too. At the end, exposure towards Cd^{2+} might cause certain forms of cancers too. As a consequence luminescent probes for aqueous phase detection of Zn^{2+} and Cd^{2+} beyond the critical limit as suggested by W.H.O. has received considerable interest where major emphasis will be to detect the analyte selectively at low concentration.

Before designing the chemosensor, the methodology of detection is prime matter of interest as the present research is emphasized towards d^{10} chemistry. Fluorescence is obviously the preferred analytical tool for detection of ionic analyte owing to its economic, portable and rapid responsive nature. In particular, d^{10} ions could facilitate chelation enhanced fluorescence (CHEF) after complextation.[20-21] The only difference between Zn^{2+} and Cd^{2+} is ionic size and relative Lewis acidity. In its consequence, tuning the cavity size in solution phase is a cumbersome process and needs several long range of synthesis. Therefore, emphasis has been levied towards the designed synthesis of chemosensor with relative level of Lewis basicity. In particular, Zn^{2+} is a hard centre and Cd^{2+} is borderline Lewis acid in nature.[22] Indeed, the chemosensors are consisted of guest analyte sensing receptor moiety which is covalently bonded to the fluorophore. The host guest sensing is converted to an optical signal by enhancement or quenching of emission. The thermodynamic selectivity is in general related to the stability of the host guest adduct which could be attributed by the suitable structural features of the host chemosensor. On the other hand optical response is related to the influence of the guest on the electronic level of the host, in general to the complexation of the guest by the host under chosen experimental conditions. Indeed thermodynamic selectivity doesn't imply necessary optical response selectivity and vice versa.[23,24] Therefore tailor made synthesis of chemosensor with proper substitution could make the suitable interaction between host and guest as per HSAB principle. In our previously reported work it has been noticed that chemosensor with tunable acidity could detect 10^{-12}M level of fluoride from aqueous specimen.[25]

As a part of our ongoing research activity in supramolecular chemistry[26-30] the present work is focused on the designed synthe-

sis of chemosensor towards selective detection of Zn^{2+} and Cd^{2+} from aqueous medium. During synthesis of the chemosensor, –OH and –NH moiety along with electron rich =N centre has been incorporated in the skeleton for rapid complexation. Owing to the borderline electronegativity among all the halogens, bromo substitution has been preferred as electron withdrawing group towards preparation of borderline chemosensor with moderate Lewis basicity. On the other hand, to prepare strong ligand field chemosensor, methoxy group has been utilized as electron donating unit. Now keeping in mind the necessity of a flexible chelator with luminescence, the chemosensor is designed with long chain molecule like tetra ethylene pentamine modified with substituted salicylaldehyde. Conjugation is not present throughout the molecule which would minimize the through bond propagation of charge, rather it would be through space effect. Through space charge transfer is generally radiative in nature. That particular radiative pathway could be altered upon cation binding and detected as fluorescent signal.

Experimentation fully supports the hypothesis and shows that Bromo substituted chemosensor is selective towards moderately strong Lewis acid Cd^{2+}. On the other hand, –OMe substituted chemosensor could selectively detect strong Lewis acid Zn^{2+} in fully aqueous medium by fluorescence turn on. TDDFT has been performed to investigate the sensing event through theoretical perspective. Moreover, UV-Vis, ^{1}H-NMR, fluorescence, FT-IR and SCXRD has been carried out in support of the experimentation. DFT has been carried out to inspect the distribution of electron density in the organic scaffold leading to the hard-soft nature of the chemosensor. It has been observed that the donating units (nitrogen of secondary amine, imine or phenolic oxygen) of –OMe substituted chemosensor are having higher charge density in comparison with Bromo substituted chemosensor. Electron pushing substituent makes the emission facile and red shifted in comparison to the electron withdrawing bromo group. The luminescence property has been further utilized for *in vitro* detection of bio benign and toxic Zn^{2+} and Cd^{2+} in aqueous medium. In case of intracellular detection of analytes, filament of a flower has been prioritized together with pollens. *This is one of the rare examples where filaments have been utilized for in vitro detection*. Actually when a flower intakes any contaminated water, it flows to the pollens through filaments. As a consequence detection of analyte from filaments would be significant for quick detection of analyte contamination. *The extracellular Zn^{2+} has been detected outside yeast cells as a biomimetic experimentation of β-cells exocytosis.* The outcome is unique of its kind and hitherto unexplored. *To the best of our knowledge, for the first time in supramolecular chemistry, the synthesized chemosensor has been utilized for detection of Zn^{2+} at 4 (±1) x 10^{-6} M concentrations from human urine specimen: a diagnostic tool for diagnosis of Pick's disease.* Utilization of flexible molecule as chemosensor to diagnose Pick's disease as a real day application is unique of its kind and certainly unprecedented.

EXPERIMENTAL SECTION

Materials and methods. All the chemicals and solvents used are of analytical grade. Solvents like methanol, water, acetonitrile, ethanol *etc* were procured from Merck India Pvt Ltd and used without purification. Metal-perchlorate salts were purchased from Merck India pvt Ltd and used as received. Tetraethylene pentamine, 5-bromo salicylaldehyde and 5-methoxy salicylaldehyde were obtained from Sigma Aldrich and used without any further treatment.

Elemental analysis has been performed in Perkin Elmer 2400C instrument. UV-Vis studies have been carried out in CARY-60. Fluorescence titration experiments have been carried out in Perkin Elmer-LS 45 machine under 10nm slit width at room temperature (298K). ^{1}H-NMR titration has been carried out in BRUKER 400MHz instrument. IR spectra (as KBr pellets, 4000−400 cm^{-1}) were taken at 298 K using a Shimadzu model 8400 S spectrophotometer.

Synthetic procedure of chemosensor 1 and 2. Tetraethylene pentamine (1 mmol, 189 mg) has been taken in 20mL ethanol in a round bottom flask under stirring condition. 3 mmol of corresponding aldehydes (605 mg, 5-bromo salicylalehyde for **1** and 456 mg, 5-methoxy salicylaldehyde for **2**) has been added into the stirring solution of TEPA and then refluxed (Scheme S1 in the SI). After 8 hours the brown semisolid mass has been collected in *vacuo* and recrystallized from ethanol. Finally the compound has been purified through methanol and ethyl acetate mixture (1:8) in silica column. The purified compound has been used for further study. Elemental analysis (%) calculated for chemosensor **1**, $C_{29}H_{32}Br_3N_5O_3$, cald. C: 47.18; H: 4.37; N: 9.49, found: C: 47.15; H: 4.36; N: 9.45. Chemosensor **2**, $C_{32}H_{41}N_5O_6$, calcd. C: 64.96; H: 6.98; N: 11.84 and found: C: 64.93; H: 6.95; N: 11.82.

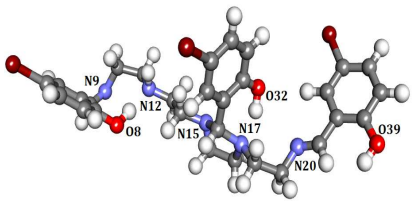
Bio-imaging study. Filament and pollens have been collected from a fresh flower. After collection of all the cells including yeast are washed with HEPES buffer. Next those are incubated in

Zn^{2+} and Cd^{2+} enriched medium (10^{-7}M) for one hour. Afterwards the cells are collected and washed with HEPES buffer. The cells are incubated in chemosensor solution for forty five minutes. Finally cells are collected and washed with HEPES buffer. The cells as obtained are taken for fluorescence imaging.

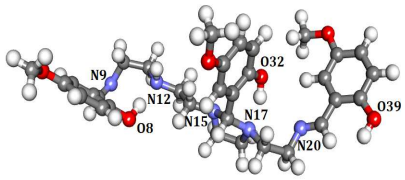
Urine sample collection and analysis. Urine specimen has been collected from local clinical laboratory under the observation of experts. It has been kept in sterilized glass bottle for further use. In chemical laboratory the urine sample has been used with batch dilution for preparation of Zn^{2+} contaminated specimen (Conc^{n}: 3-5 x 10^{-6}M). Experimentation has been repeated twice with each concentration. Best result has been obtained at Zn^{2+} concentration 5 x 10^{-6} M. After experimentation all the urine samples have been discarded in sanitary chamber. The gloves and other related items have been discarded following the proper protocol.

RESULTS AND DISCUSSION

DFT Study for obtaining Loewdin spin population analysis: strong and moderate donor chemosensor. The chemosensor **1** and **2** have been synthesized with variable electronic substitution. The electron donating –OCH_3 group of chemosensor **2** makes the skeleton electronically richer than **1**, which in turn makes the chemosensor **2** strong ligand field donor. *Loewdin* spin population analysis of both the chemosensors has been performed by using density functional theory (DFT) (Table S1-S4 in the SI).[31] It has been observed that the donor centres (N9, O8, N12, N15, N17, O32, N20 and O39) of chemosensor **2** are more electron rich than chemosensor **1** (Figure. 1). Furthermore, the HOMO-LUMO energy gap of chemosensor **2** **(ΔE= -4.0392eV)** is lower than **1** **(ΔE= -4.3854eV)** which makes the emission more facile for chemosensor **2** (*vide* supra, fluorescence titration section). Interestingly, the nature of electronic distribution in HOMO and LUMO for both the chemosensors is nearly similar (Figure. 1).



N9: -0.473 **O8:** -0.582
N12: -0.595 **N15:** -0.522
N17: -0.531 **O32:** -0.519
N20: -0.451 **O39:** -0.492



N9: -0.479 **O8:** -0.593
N12: -0.598 **N15:** -0.522
N17: -0.531 **O32:** -0.530
N20: -0.456 **O39:** -0.508

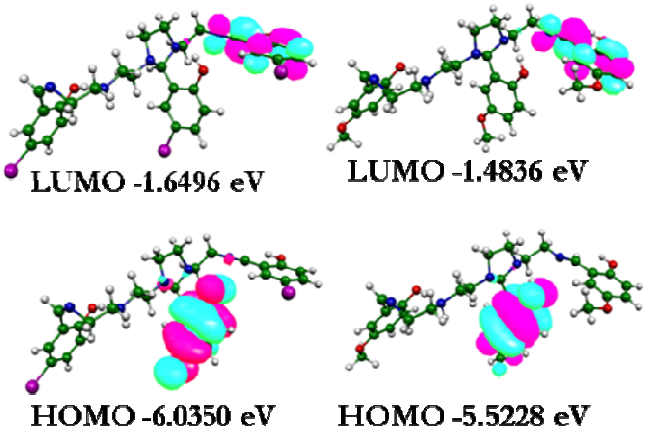


Figure. 1 Geometry optimized structure of chemosensor **1** and **2** with *Loewdin* spin population of selective donor centre, HOMO-LUMO orbital plot with energy.

Absorption study of the chemosensors has been performed in acetornitrile medium at 10^{-4}M concentration. Titration of the chemosensor **1** and **2** with two competitive ions, *i.e.;* Zn^{2+} and Cd^{2+} has been carried out at 10^{-4}M concentration. The experiment reveals that the affinity of interaction between chemosensor **1** is higher for Cd^{2+} than Zn^{2+} (Figure S1, in the SI). On the other hand, in case of chemosensor **2**, owing to its electron rich binding sites the interaction is stronger for Zn^{2+} over Cd^{2+} (Figure S1, in the SI). Indeed the affinity of the chemosensor towards the competitive ions has been established.

Indeed, fluorescence and absorbance both are useful sensing tools. However, fluorescence is more sensitive than absorbance technique. The absorbance is measured by the intensity difference between the light passed through reference and sample. On the other hand fluorescence intensity is measured directly without comparing with any reference specimen.[32] Indeed detection of low level of light and electronic impulses due to single photons are measurable with most photomultiplier tubes. Thus fluorescence is used for trace level detection. Indeed with modern electronic set up and updated optical instrument it is difficult to measure small amount of absorbed light. Even if the set up allows detecting low optical density however cuvettes might interfere.[32] Furthermore, fluorescence measurement at low concentration is possible owing to the dark background in fluorescence study which does not happen in absorbance. Therefore fluorescence has to be performed for trace level detection of the biologically significant and chemically almost similar inorganic metal ions, *i.e.;* Zn^{2+} and Cd^{2+}.

Recognition of Cd^{2+} and Zn^{2+} by luminescence with 10^{-12}M detection limit. Upon photo-excitation at 325 nm and 350 nm chemosensor **1** and **2** exhibits fluorescence at 430 nm and 445 nm respectively (5 x10^{-6}M) in acetonitrile solvent (pH: 7.4, HEPES buffer). Detection of biologically relevant metal ion has been carried out in 100% aqueous medium. All the solution of metal perchlorate (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}) salts have been prepared at 5 x 10^{-6}M concentration in water. Initially the response of the chemosensors has been checked under naked eye observation with UV light irradiation. In case of **1** and **2** upon addition of different cations, fluorescence turn on signal has been noticed in presence of Cd^{2+} and Zn^{2+} (Figure. 2).

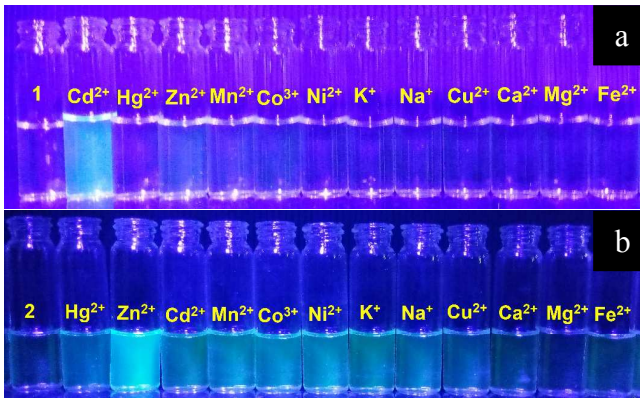


Figure. 2 Fluorescence response of (a) **1** and (b) **2** in presence of cations (pH of the medium 7.4).

During fluorescence titration chemosensor **1** has shown fluorescence turn on with gradual addition of Cd^{2+} at just 0.012 equivalents. The interesting observation is that upon addition of just 0.012 equivalent leads to huge enhancement. Even all the chemosensor molecule also couldn't bind with Cd^{2+}, *i.e.;* chances of further fluorescence enhancement is there too. Actually the adduct formed between chemosensor **1** and Cd^{2+} is so fluoresce that reached to saturation of emission immediately (Figure 3i, saturation of emission is at 1000 a.u. of intensity in LS-45, Perkin Elmer instrument).

In case of chemosensor **2** addition of Zn^{2+} in water up to 2 equivalents leads to fluorescence turn on (Figure. 3ii). Other cations and solvent water doesn't cause any such enhancement within the same limit of addition (Figure 3iii and iv). Jobs plot reveals 1:2 binding between chemosensor and analyte in each case (Figure S2 in SI). The limit of detection for Cd^{2+} is 500 pico mole and for Zn^{2+} it is 5 ppb which is far beyond the critical limit as suggested by W.H.O.

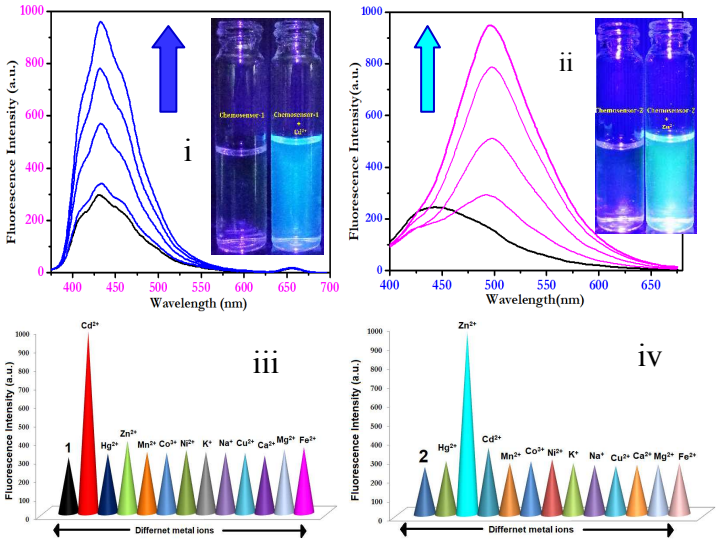
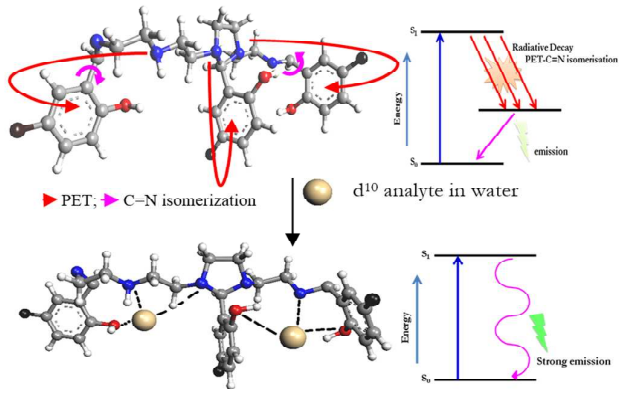


Figure. 3 Fluorescence titration of (i) **1** and (ii) **2** in presence of Cd^{2+} and Zn^{2+} (pH: 7.4, HEPES buffer), (iii) chemosensor **1** + cations and (iv) chemosensor **2** + cations.



Scheme 1. Transition in chemosensors and fluorescence turn on after detection of cations.

The drastic fluorescence enhancement of the chemosensor in presence of cations could be explained by PET-CHEF-C=N isomerisation restriction mechanisms. The chemosensors are having photoinduced electron transfer from electron rich nitrogen (secondary amine or imine) to phenyl ring which causes inherent emission of the system at lower extent. Secondly the isomerisation around C=N bond is an important issue for dissipating the excited state energy of the chemosensors (Scheme 1). Upon addition of Cd^{2+} and Zn^{2+} in chemosensor **1** and **2** solution it becomes a perfect match in terms of HSAB principle which leads to complexation of the ionic analyte with chemosensors. The complexation has restricted the PET which leads to fluorescence enhancement. On the other hand, the chelation of cations by chemosensors

causes chelation enhanced emission (CHEF) towards drastic enhancement of fluorescence intensity. Furthermore, complexation makes the restriction of the isomerisation around C=N bond which in turn makes the chemosensors rigid and ultimately leads to turn on emission (Scheme 1). Time dependent density functional theoretical (TDDFT) calculation has been performed for chemosensor **1** and **2** with the corresponding analytes, *i.e.;* Cd^{2+} and Zn^{2+}(figure S3, in the SI). Indeed the chelation effect of the chemosensor towards Zn^{2+} and Cd^{2+} is established which has resulted in CHEF. As a consequence, a combined mechanism towards fluorescence turn on by PET-CHEF-C=N isomerisation based pathway is described with the aid of fluorescence, TDDFT and SCXRD. In both cases, PET-CHEF-C=N isomerisation pathways are similar. However for Zn^{2+} the turn on sensing is going through charge transfer pathway too. There is a clear swing of emission spectrum from 445 nm to 500 nm has been observed. This swing is in line with charge transfer within the molecule. Owing to its hard Lewis acid nature Zn^{2+} may initiate the ILCT (intraligand charge transfer) in the complex formed with chemosensor **2**. Lifetime of charge transfer state is high in general, which perhaps is the reason behind comparatively slow response of the chemosensor **2**. In particular, during fluorescence titration it has been observed that upon addition of **2** equivalents of Zn^{2+} the fluorescence has been enhanced whereas for chemosensor **1** just addition of 0.012 equivalent is sufficient. TCSPC has been performed for chemosensor 2 and its complex with Zn^{2+} which are found to be 2.72 and 2.84 ns respectively. Indeed, the enhancement due to charge transfer phenomena is established. (Table S5, SI for details). It might be a reason that ILCT is slowly responding and as a consequence addition of 2 equivalents of Zn^{2+} became necessary. However, finally the ILCT makes the fluorescence facile and thus it has been shifted to lower energy. Therefore, fluorescence of chemosensor **2** with Zn^{2+} follows PET-CHEF-C=N isomerisation-ILCT pathway. Interestingly the binding of chemosensor **1** with Cd^{2+} is reversible with CN^- for four cycles. On the other hand, in case of chemosensor **2** and Zn^{2+} adduct, the chelation is not reversible with CN^-. However, with EDTA the chelation of chemosensor **2** and Zn^{2+} is reversible for three cycles.

Interaction of chemosensors with analyte in solid phase: FT-IR analysis. To investigate the interaction between both the chemosensor **1** and **2** in presence of d^{10} analytes in solid phase, FT-IR has been carried out (Figure. 4). The characteristic vibrational stretching of chemosensor **1** and **2** are as follows: C-N: υ 1273cm^{-1} (chemosensor **1**) and 1270 cm^{-1} (Chemosensor **2**); C=N: υ 1628cm^{-1} (chemosensor **1**) and 1637 cm^{-1} (Chemosensor **2**); C-O: υ 1178cm^{-1} (chemosensor **1**) and 1160 cm^{-1} (Chemosensor **2**); aromatic C=C: υ 1480 cm^{-1} (chemosensor **1**) and 1495 cm^{-1} (Chemosensor **2**). Imine bond in the range of 1628-1637 cm^{-1} swings to 1632 and 1610 cm^{-1} for chemosensor **1** and **2** after complexation. FT-IR stretching at 1273 and 1270 cm^{-1} has been shifted to 1277 and 1275 cm^{-1}. The swing in stretching frequency reveals that chemosensor interacts with the analyte in solid phase as well.[33]

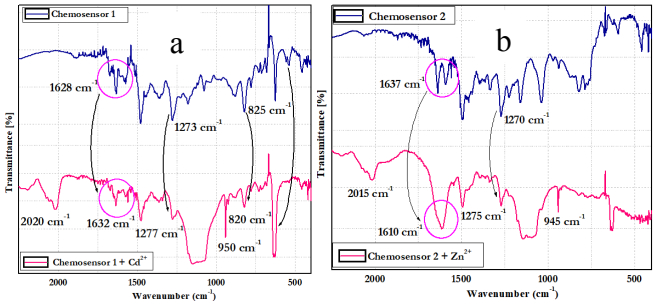


Figure. 4 FT-IR analysis of (a) **1** and (b) **2** in presence of 2 equivalents Cd^{2+} and Zn^{2+}.

^{1}H-NMR titration of the chemosensor with Zn^{2+} and Cd^{2+}. ^{1}H-NMR titration of chemosensor **1** and **2** with Cd^{2+} and Zn^{2+} has been carried out in acetonitrile-d_3 solvent. The chemosensor shows the expected chemical shift for the skeletal protons (blank spectrum of the chemosensor **1** and **2**, Figure S4 in SI). Indeed the –OH protons are labile and thus couldn't be located easily. However the blank spectrum of the chemosensor **1** and **2** shows –OH proton signals at ~13.33 and ~12.67 ppm. Indeed, Loewdin spin population as performed by DFT calculation (*vide* supra, figure 1) suggest that the electrons of chemosensor **1** would be less shielded than chemosensor **2**. Thus the -OH signals of chemosensor **1** has been found at downfield region in comparison with chemosensor **2**. The –NH proton for both the chemosensors has probably been observed within 2-3 ppm. In particular, due to overlapping of chemical shift with $–CH_2$ proton the exact location could not been located. The magnified view of 6-9 ppm range has been placed in supporting information (figure S5, in the SI). Cations are taken in water-d_2. In case of both the chemosensors, addition of d^{10} analytes has shown downfield shifting of the skeletal protons (Figure. 5).

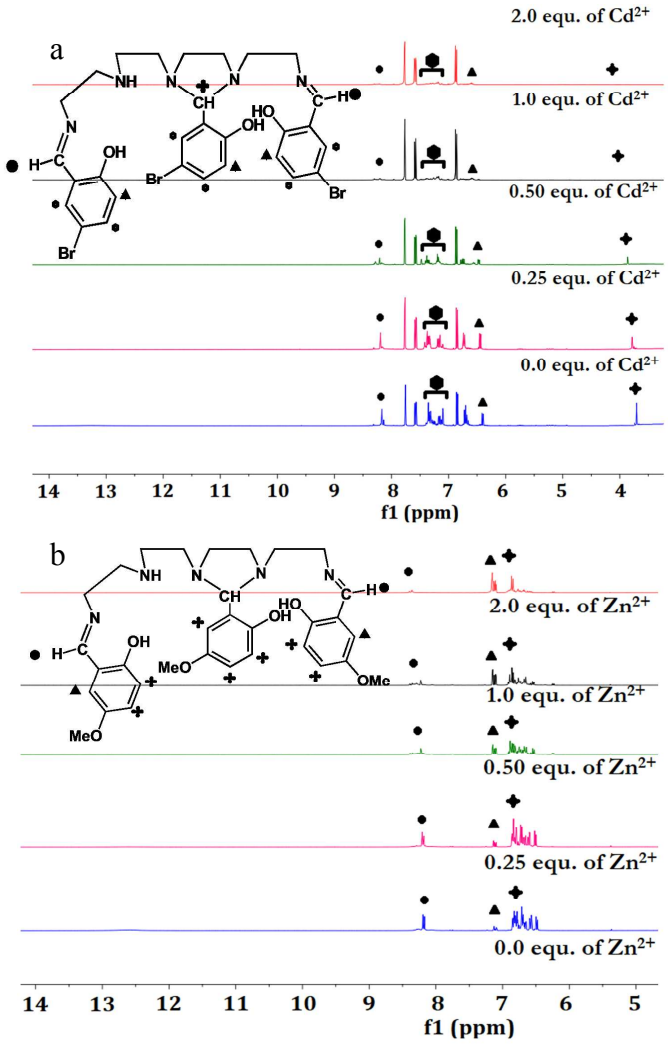


Figure. 5 ^{1}H-NMR titration of (a) **1** and (b) **2** in presence of Cd^{2+} and Zn^{2+} respectively.

Figure 5a depicts the ^{1}H-NMR titration of chemosensor **1** with Cd^{2+}. Among different protons, the imine protons at ~8.2 ppm shows downfield shifting and gradually minimized. After 2 equivalents of Cd^{2+} addition, the imine protons are almost completely diminished which suggests the complexation of the chemosensor with analyte by possible interaction with imine nitrogen (*vide* infra, SCXRD analysis). Three adjacent protons of phenolic group at ~ 6.5 ppm along with the skeletal protons are shifted downfield after gradual addition of Cd^{2+}. Similarly in case of chemosensor **2** with Zn^{2+}, the imine protons at ~8.3 ppm and other skeletal protons have been shifted to downfield after analyte detection. It goes without saying that the interaction between organic chemosensor and cationic analyte will obviously take place through imine nitrogen and phenoxyl oxygen which has been observed during NMR titration. Furthermore, binding of cationic analyte will result in downfield shifting of the other conjugated skeletal protons, which could be observed from the ^{1}H-NMR titration as well.

In particular, solution state properties might not be always reproduced by solid state characterization. However keeping in mind the significance of the present work in the relevant field, single crystal X ray study has been prioritized. As per thermodynamics, two precursors (chemosensor and cationic analyte herein) when interacted together and crystallized, the entropy could decrease from the perspective of translational and rotational degrees of freedom. Therefore thermodynamically the process would not be favourable. In the present case, the working solvent plays a key role about crystallization. Indeed, solvent molecules could balance the loss of entropy by getting trapped or released. Our careful study about crystallization of chemosensors with the specific analytes in acetonitrile and water medium results in microcrystalline product, which are not suitable for single crystal X ray analysis. Afterwards, the crystallization has been attempted with the same solvent mixture (acetonitrile and water) in presence of another third solvent such as di methyl formamide (DMF), toluene *etc*. However no such single crystal suitable for X ray study has been obtained. Indeed other solvent mixtures apart from acetonitrile/water have not been attempted as emphasis is to obtain crystal from the same solvent mixture where the sensing study has been performed (*vide* supra, figure 2-3). In its consequence a new compound has been synthesized keeping the same skeleton without any substitution (*i.e.;* -Br and -OMe). Crystallization has been attempted in acetonitrile/ water medium with addition of small amount of DMF, toluene as third solvent. Zn^{2+} and Cd^{2+} have been added to the newly synthesized compound gradually and the ratio has been maintained similar as performed in case of titration studies for the chemosensors. Crystals thus obtained were analysed by SCXRD study which reveals the co-crystallization of DMF and water which balances the lost entropy and certainly favors the crystallization process. Moreover one water molecule has been coordinated with central metal ion towards fulfilling the geometry as well as the thermodynamics. ORTEP of the Zn^{2+} adduct reveals that two metal ions have been ligated among which one is hexa coordinated and another centre is penta coordinated. O3 and O1, two phenolic oxygens have been bonded in μ_2 fashion. The single crystal x ray analysis reveals 1:2 (host:guest) complexation which in turn supports the Jobs plot analysis performed during fluorescence titration (figure S2, in the SI). Molecular view of the crystal structure is shown in figure S6, in the SI.

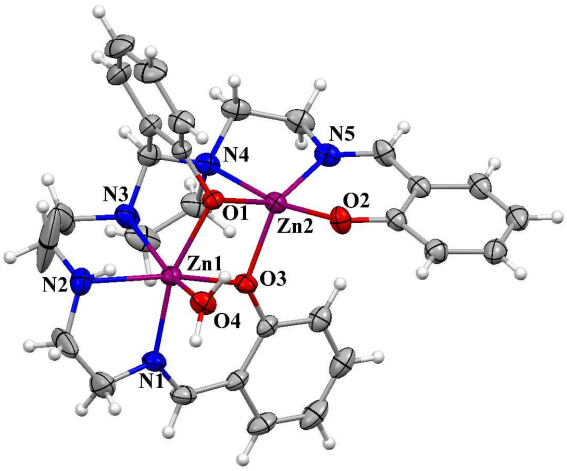


Figure 6. Binding mode of the representative of the chemosensor **1** and **2** with one of the representative of d^{10} analyte, Zn^{2+} herein.

Intracellular detection of Cd^{2+} and Zn^{2+} from androecium: application in bio imaging. Alzheimer's disease is a neurodegenerative disorder which includes enhanced levels of Zn^{2+} within amyloid plaques. This particular ion becomes excessive in intracellular area which contributes to neurodegeneration in epilepsy. In most mammalian cells intracellular level of Zn^{2+} is in the range of ~100–500 μM.[34] However, human cells are having 200-300 μM level of Zn^{2+}. On the contrary, in case of Cd^{2+} the presence of this ion in cell membrane would cause various diseases and could damage the cell structure. Therefore, it is obvious to develop a smart chemosensor like **1** and **2** for selective recognition of Cd^{2+} and Zn^{2+} in biological aqueous medium.

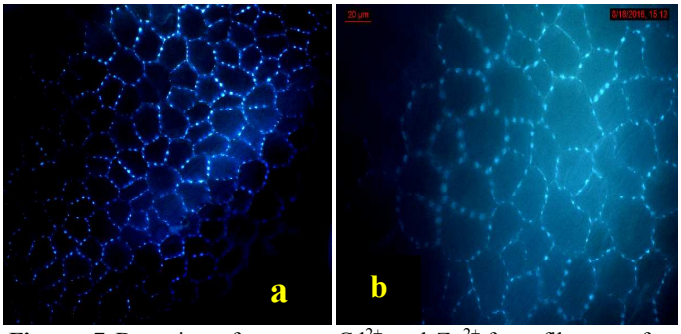


Figure. 7 Detection of aqueous Cd^{2+} and Zn^{2+} from filament of *Tecoma Stans* by (a) **1** and (b) **2** respectively incubated in 35°C.

In every case these analytes enter into body through water and causes various diseases. As a consequence, detection of the analytes from different living cell is highly essential. The major chances of the contamination are associated through ground water system. In view of above, industrial waste water has been collected from Durgapur, West Bengal surroundings. After primary filtration the water sample has been used for artificial preparation of the contaminated specimen with Zn^{2+} and Cd^{2+} by batch dilution at 10^{-7}M concentration. In general, intracellular detection is studied inside pollens only. However the present work has been carried out inside the androecium, *i.e.;* both the anther and filament of a flower. The slender stalk, *i.e.;* filament holds the anther which produces pollens. Pollens and filament have been collected and initially incubated in analyte spiked water for 1 hour to ensure proper uptake of the cations. Next, the pollens and filaments are washed with HEPES buffer to ensure maximum cell survivability. Finally those are incubated in the chemosensor solution at 5 x 10^{-6}M concentration. Within half an hour the pollens and filaments are taken outside and washed with HEPES to avoid any background emission caused by externally settled chemosensors. It has been observed that for Cd^{2+} the cells and filaments are sparkling with blue emission (Figure. 7.8 a), whereas for Zn^{2+} the cyan emission has been observed (Figure. 7,8 b) which are in line with fluorescence titration outcomes (*vide* supra).

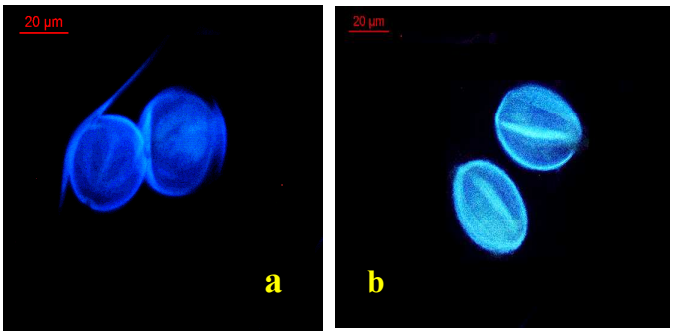


Figure. 8 Detection of Cd^{2+} and Zn^{2+} from pollens grains of *Tecoma Stans* by (a) **1** and (b) **2** respectively incubated in 35°C.

Intracellular and extracellular detection of Zn^{2+} from yeast cells: application in type 1 and type 2 diabetes. Zn^{2+} supplementation is directly related with the improvement of glycaemic control. Indeed, research work has established that Zinc stimulates glycolysis with inhibition of gluconeogenesis. Furthermore it increases the activity of glycolytic enzymes, phosphofructokinase and pyruvate kinase.[17] Reports suggest that Zn^{2+} concentration in the pancreas is reduced upto 50% in case of diabetic patients compared to non diabetic. Actually, insulin is stored as a hexamer bonded with Zn^{2+} in beta cell which is released by exocytosis in response to stimuli such as glucose. During exocytosis the Zn^{2+} bonded insulin get dissolved and dissociates by releasing Zn^{2+} and insulin. Furthermore the Pancreatic β-cells have high level of Zn^{2+} content (~10–20 mM). Therefore detection of Zn^{2+} efflux would allow the performance of beta cell together with monitoring diabetes mellitus and intracellular detection would help to diagnose the Zn^{2+} level in β-cells. In view of above emphasis has been giv-

en towards extracellular together with intracellular detection of Zn^{2+} in yeast. Yeast cells are primarily washed with HEPES buffer solution. Next those have been incubated in Zn^{2+} contaminated water solution at different concentration (2 x 10^{-6}M for Figure. 9a and 5 x 10^{-6}M for Figure. 9b). After one hour of incubation the cells are then incubated in chemosensor solution. Finally after 45 minutes the cells are taken out and washed with buffer. Cells are visualized under fluorescence microscopy. It has been observed that the yeast cells and medium both are sparkling with emission for Zn^{2+} (Figure. 9).[34]

The study is biomimetic experimentation towards exocytosis of β cell of pancreases. Actually Zn^{2+} is necessary for stability of insulin together with protection of against oxidative stress in type 1 and type 2 diabetes with their related pathologies. The initiative in this avenue is unique of its kind.

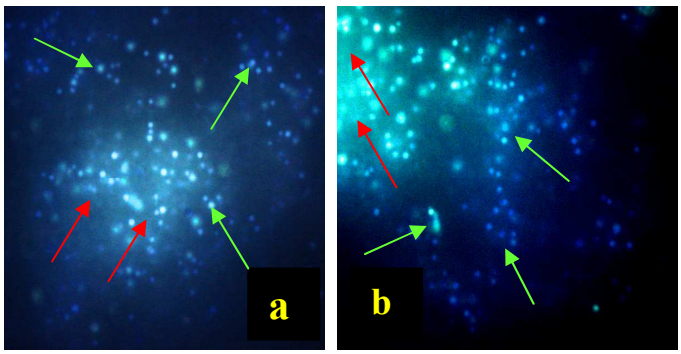


Figure. 9 Detection of Zn^{2+} from yeast cells by chemosensor **2** at different concentration (a) 2 x 10^{-6}M and (b) 5 x 10^{-6}M (incubated in 35°C, red arrow represents extracellular detection; green arrow represents intracellular detection).

Biosensor: diagnosis of *Pick's disease* from urine. In general chemosensor for detection of cationic analyte is reported in literature. However the major issue is related with the practical utility of the chemosensor, because in most of the cases the analyte detection occurs in semi aqueous medium. A very few chemosensors are known for analyte detection from aqueous specimen; however the limit of detection is not low. Significantly, Zn^{2+} detection from human urine specimen beyond 7.6 x 10^{-6} M level indicates the possibilities of *Pick's disease*. To the best of our knowledge no such evidence is found towards detection of Zn^{2+} from human urine specimen as a diagnosis of *Pick's disease.* It is well known that *Pick's disease* causes an abnormal enhancement of Zn^{2+} in human blood and urine. As the normal level of Zn^{2+} in urine is 7.6 x 10^{-6}M therefore it could be presumed that beyond this concentration is not safe. Fluorescence titration reveals that chemosensor **2** could detect 3-5 x 10^{-6} M concentration of Zn^{2+} from aqueous medium. As a consequence chemosensor **2** is applied for detection of Zn^{2+} from urine specimens. A series of urine specimens have been collected from local clinic and artificially Zn^{2+} has been added into urine specimen by batch dilution (concentration of Zn^{2+}: 3-5 x 10^{-6}M). It is worthy to mention that the chemosensor **2** (5 x 10^{-6}M in acetonitrile, pH 7.4, HEPES buffer) doesn't show any response to the pure urine specimen. When the Zn^{2+} containing specimen has been added to the chemosensor solution it shows fluorescence turn on in line with the fluorescence titration outcome (*vide* Figure. 3ii). In each case of urine sample the experimentation has been carried out twice (see the experimental section for details). The result indicates that as a biosensor the chemosensor **2** could be applied for diagnosis of abnormal enhancement of Zn^{2+} in urine, *i.e.; Pick's disease* (Figure. 10).

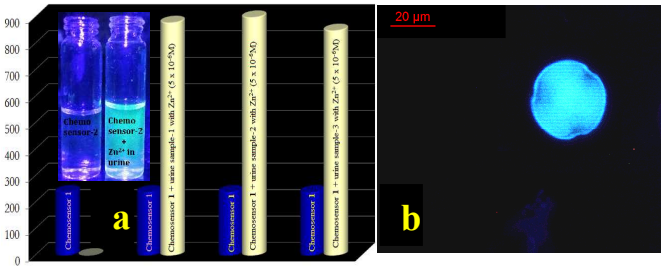


Figure. 10 (a) Detection of Zn^{2+} from urine specimen by chemosensor **2**; (b) *in vitro* detection of Zn^{2+} from pollen grains of *Tecoma Stans* under fluorescence microscopy at 35°C.

The successful detection makes us curious about further exploration of diagnosis of *Pick's disease*. Now, urine sample has been used as medium for intracellular detection of Zn^{2+}. Instead of using water medium for incubation of pollen cells, urine specimen has been used as medium. Pollens have been incubated in artificially spiked Zn^{2+} ($conc^{n}$: 5 x 10^{-6}M) containing urine specimen used for *Pick's disease* diagnosis study. Later on it has been washed with HEPES buffer. Finally the cells are incubated in chemosensor **2** solution for 45 minutes and taken for fluorescence imaging. The cells are sparkling with cyan emission and reveals that intracellular Zn^{2+} coming from urine specimen have been detected successfully by biosensor **2** (Figure. 9b).[35] As a consequence it could be concluded that the chemosensor could be used as biosensor. In this relevance, Table 1 depicts the comparison of the present work with the recently reported chemosensors. Altogether the present work to the best of our knowledge represents

for the first time a real day applicable chemosensor to diagnose human *Pick's disease* from body fluid.

CONCLUSIONS

In conclusion, two chemosensors have been precisely synthesized by varying the substitution. Electron withdrawing bromo group containing chemosensor could detect borderline Lewis acid Cd^{2+} by blue emission. On the other hand, methoxy like electron donating substitution makes the chemosensor strong donor and eventually detects strong Lewis acid Zn^{2+} by fluorescence turn on. Interestingly strong Lewis acid Zn^{2+} induces charge transfer inside the chemosensor and follows PET-CHEF-C=N isomerisation-ILCT pathway for fluorescence enhancement. On the other hand, Cd^{2+} follows PET-CHEF-C=N isomerisation with limit of detection 500 pico mole.

Intracellular detection of both Zn^{2+} and Cd^{2+} has been carried out with androecium (both filament and pollens) using industrial waste water as medium. Furthermore, Zn^{2+} and Cd^{2+} detection has also been carried out inside yeast cells too. The phenomenal finding of the present work for the first time in the history of supramolecular chemistry is to detect Zn^{2+} from urine, *i.e.;* diagnosis of *Pick's disease*. One step ahead Zn^{2+} has been detected from the pollen cells incubated in urine specimen contaminated with Zn^{2+}. The chemosensor could be useful as diagnostic tool for *Pick's disease*. Epidemiological investigations suggest that the Zn^{2+} concentration in human body might be related with diabetes. The extracellular Zn^{2+} has been detected outside yeast cells as a bio-mimetic experimentation of β-cells exocytosis. Our group is actively engaged in development of next generation therapeutic agent based on designed synthesis of Zn^{2+} selective chemosensor towards diabetes like worldwide endemic together with *Pick's disease*.

ASSOCIATED CONTENT

Supporting Information. Details of the synthetic scheme, coordinates for DFT calculation, Loewdin spin population analysis in tabular form, UV-Vis titration curve of chemosensor **1** & **2** with Cd^{2+} and Zn^{2+}, basic ^{1}H-NMR of chemosensor **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

Table 1 comparison of the present work with other reported chemosensor.

Sl. No.	Medium of Detection	Limit of Detection	*In vitro* Sensing	Detection from filament	Pick's diagnosis	Bio-mimetic of exocytosis of β cells for diabetes	Ref
1.	Organic Solvents	2.3 pM	Yes	No	No	No	36
2.	Simulated physiological medium	5 – 100 μM	No	No	No	No	37
3.	Aqueous Acetonitrile solution	4.0×10^{-8} M.	Yes	No	No	No	38
4.	Aqueous medium	Not found	Yes	No	No	No	39
5.	Aqueous buffer solution	0.1 μM	No	No	No	No	40
6.	Ethanol	Not found	No	No	No	No	41
7.	MES buffer containing 1% Ethanol	0.18 μM	Yes	No	No	No	42
8.	Actonitrile	0.35 μM	No	No	No	No	43
9.	CAPS Buffer	4.5×10^{-9}	No	No	No	No	44
10.	Tris buffer	Sub micromolar	No	No	No	No	45
11.	EtOH – H_2O	3×10^{-8}	Yes	No	No	No	46
12.	Acetonitrile	Not found	No	No	No	No	47
13.	Ethanol	570±10 ppb	No	No	No	No	48
14	CH_3CN:HEPES (1:1)	Not found	Yes	No	No	No	49
15	Aqueous	60 nM	Yes	No	No	No	50
16	Mixed Aqueous	56 ppb	No	No	No	No	51
17	CH_3CN: Buffer (1:1)	Not found	No	No	No	No	52
18	Aqueous	2.5×10^{-6} M	Yes	No	No	No	53
19	DMSO:H_2O (1:9)	3.5×10^{-8} g/L	Yes	No	No	No	54
20	**100 % water**	**5×10^{-9} M**	**YES**	**YES**	**YES**	**YES**	**Present Work**

AUTHOR INFORMATION

Corresponding Author

** E-mail: *pr_banerjee@cmeri.res.in* & *priyabrata_banerjee@yahoo.co.in* Webpage: *www.cmeri.res.in* & *www.priyabratabanerjee.in* Fax: +91-343-2546745; Tel: +91-343-6452220.

* E-mail: *drswapan63@gmail.com* Tel: +91-9434042985.

[±] Present address of Dr Pritam Ghosh: Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, Technion City, Haifa 3200008, Israel.

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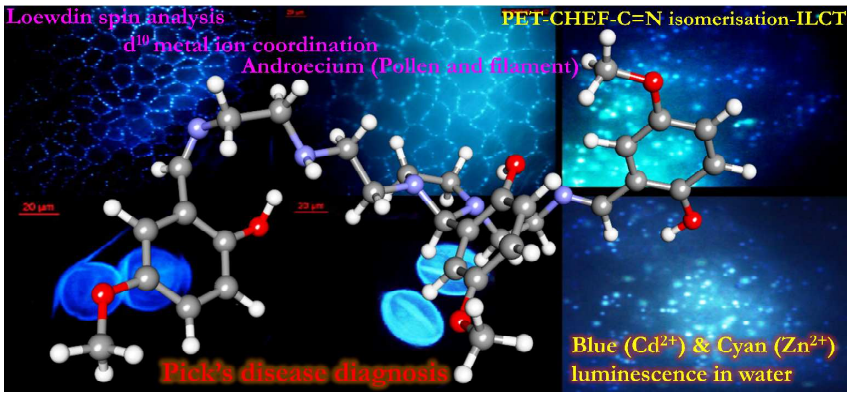
Loewdin spin analysis
d10 metal ion coordination
Androecium (Pollen and filament)
PET-CHEF-C=N isomerisation-ILCT
Pick's disease diagnosis
Blue (Cd2+) & Cyan (Zn2+)
luminescence in water