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Hydrazide based novel selective and sensitive optical chemosensor^{View Article Online} for the detection of Ni²⁺: Applications in living cell imaging, molecular logic gate and smart phone

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Abstract

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A novel flexible "N", "O" rich hydrazide based Schiff base chemo-receptor 2-(benzamido)-N'-((pyridin-2-yl)methylene)benzohydrazide (L) has been designed, synthesised and characterised by ¹H-NMR, IR spectroscopy, ESI-MS spectrometry and single crystal XRD analysis. The receptor showed colorimetric and fluorometric "*turn on*" response in presence of only Ni²⁺ ion in methanol-*tris*-HCl buffer medium (10 mM, pH 7.2, 1:1 v/v). The jobs plot analysis revealed that receptor forms 2:1 complex with Ni²⁺ ion, further confirmed by ESI-MS analysis. The single crystal structure of L-Ni²⁺ complex (**2**) has also been isolated. The detection limit was calculated to 1.8 μ M colorimetrically and 1.18 μ M fluorometrically, which are far lower than those recommended by WHO guidelines for drinking water. The experimental results have further been supported by the DFT studies. The chemosensor L can be applied to the recovery of contaminated water samples, building of molecular logic gate, smart phone and living cell imaging.

Keywords: quinazolinone / Schiff base/ colorimetric sensor / fluorometric sensor / Ni²⁺ sensor /cell imaging/ logic gate.

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Introduction

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In supramolecular chemistry, the design and construction of efficient chemo-receptor which can precisely detect and quantify heavy transition metal ions, are highly focused in current research interest, as these ions have significant role in biological, environmental and industrial processes.¹⁻⁴ In earlier few years, several traditional analytical techniques like inductively coupled plasma emission spectroscopy (ICP-ES)⁵, chromatography,⁶ potentiometry,⁷ atomic absorption spectroscopy (AAS)⁸ and flow injection amperometry;⁹ were mainly used for the detection of heavy metal ions but most of these techniques involved major drawbacks in terms of selectivity, sensitivity, time consuming, troublesome sample preparation, skilled personnel and also use of expensive and sophisticated instruments.¹⁰ Among the alternative convenient approaches, optical signalling based chemosensors have been used as promising tools in recent times, because of their operational advantages, real and onsite analysis, high selectivity and sensitivity.¹¹⁻¹³

Generally, the optical chemosensors for metal ions can be designed by the combination of binding sites and the signalling units through a covalent linker where the receptors are selective for specific metal coordinations, which respond as optical signal through signalling subunit.¹⁴ The specific ion selectivity depends on the relative stability of the host guest interaction, hence can be influenced by the cavity size, flexibility of chelator and hardness of the coordinating atoms; whereas the optical response is due to influence of guest on the electronic environment of chromo-fluorophore.¹⁵ As the guest selective binding not always produce proper signal, thus tailor made synthesis of chemoreceptor with desired functionalities is necessary to achieve measurable signal through change in emission and/or absorption upon coordination.

Due to proper geometrical and electronic arrangement, Schiff base (>C=N) organic framework integrated with a chromophoric and/or fluorophoric unit is one of the most promising tools for optical based metal ion sensing.^{16,17} The ligand metal charge transfer (LMCT) along with intermolecular charge transfer (ICT) and chelation enhancement fluorescence effect (CHEF) play vital roles in designing Schiff base sensor which can alter the intensity and wavelength of absorption and emission bands. Moreover fluorescence turn on process has great advantages in comparison to turn off sensor, as ease of low level measurement from its dark background which reduce false pulsing and increasing sensitivity.¹⁸

Among the transition metal series, Ni²⁺ is an essential constituent of several metaloenzymes like hydrogenases, carbon monoxide dehydrogenases and acireductone dioxygenases

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and has crucial role in biological processes such as metabolism, respiration and biosynthesis and the control of the second seco

Motivated by the above facts and in continuation of our ongoing research work,^{27,28} we have designed and synthesized a novel Schiff base chemosensor N'-(2-hydroxybenzylidene)-2-(benzamido) benzohydrazide (**L**), by the coupling of synthesized amine, 3-amino-2-phenyl-4(3H)-quinazolinone (**1**) with 2-pyridene carboxaldehyde. The background strategy behind the designing of chemoreceptor is mainly based on three ideas. At first, the flexibility of the chemosensor would permit coordination of suitable metal cations. Secondly presence of multiple chromophoric moiety >C=O and >C=N may produce proper visible signal. Finally, multiple –CONH- moieties can increase water solubility of the proposed receptor (**L**). The proposed chemosensor (**L**) can selectively detect Ni²⁺ ion both colorimetrically and fluoremetrically in aqueous medium. The recognisation process was further confirmed by the DFT studies and isolation of single crystal of the metal complex. Moreover the chemosensor has successful application in real sample analysis, living cell imaging, smart phone base analysis and in building of molecular logic gates.

Experimental

Materials and General information

All the required materials used for synthesis were obtained from Sigma-Aldrich and used as received. The analytical grade solvents used for the overall experiments and freshly prepared double deionized water was used for dilution purpose and preparing *tris* HCl buffer (10 μ M, pH=7.2) solution. The metal ion solutions were prepared from their nitrate salts except sulphate salt of Mn²⁺ and Fe²⁺ and potassium tetrachloroplatinate(II) salt for Pt²⁺. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker DRX spectrometer operating at 400MHz in CDCl₃solvent and chemical shift were recorded in ppm relative to TMS. Absorption spectra were recorded on a Shimadzu UV 1800 spectrophotometer using 10 mm path length quartz cuvettes with the wavelength in the range of 200-800 nm. High resolution mass (HRMs) spectra were recorded on a Waters mass spectrometer using mixed solvent HPLC methanol and

triple distilled water. The pH measurements were done using a digital pH meter $Mere Market Online Control adjusting dilute hydrochloric acid and sodium hydroxide in buffer solution. Solutions of the receptor L (<math>1 \times 10^{-5}$ M) and metal salts (1×10^{-4} M) were prepared in methanol-*tris*-HCl buffer (10 mM, pH 7.2) medium (1:1 v/v) and H₂O respectively.

X-ray data collection and structural determination

X-ray single crystal data were collected using MoK α ($\lambda = 0.7107$ Å) radiation on aBRUKER APEX II diffractometer equipped with CCD area detector. Data collection, datareduction, structure solution/refinement were carried out using the software package of SMART APEX.²⁹ The structures were solved by direct methods (*SHELXS-97*) and standard Fourier techniques, and refined on *F2* using full matrix least squares procedures (*SHELXL-97*) using the *SHELX-97* package³⁰ incorporated in *WinGX.*³¹ In most of the cases, non-hydrogen atoms were treated anisotropically. Hydrogen atoms were fixed geometrically at their calculated positions following riding atom model. Structural information of **L** and **2** have been deposited at the Cambridge Crystallographic Data Center (CCDC numbers 1917470 and 1917471 respectively).

Synthesis of N-(2-hydroxybenzylidene)-2-(benzamido)benzohydrazide (L)

The synthetic procedure and single crystal structure of the compound 1 (3-amino-2-phenyl-4(3H)-quinazolinone) has already been reported by us elsewhere.³² The Schiff base chemoreceptor L was synthesized by preparing individual solution of 3-amino-2-phenyl-4(3H)quinazolinone (1, 1.185 gm., 5mmol) and 2 pyridine carboxaldehyde (0.535 gm, 5 mmol) in dehydrated methanol followed by mixing of these two solution in presence of catalytic amount of glacial acetic acid. The reaction mixture was refluxed for 2h maintaining dry condition. The colourless clear solution was kept in air for 24h. Crystalline solid separated out was filtered, washed with methanol and dried in air. Yield, 1.46 g, 85 %; m.p.>200°C. Single crystals suitable for X-ray analysis were obtained from the slow evaporation of methanol solution of L. Anal. Calc. for C₂₀H₁₆N₄O₂: C, 69.76; H, 4.68; N, 16.27. Found C, 69.68; H, 4.63; N, 16.21%. EI-MS: m/z 367.13 (M+Na⁺, 100%) (Fig. S1). FTIR/cm⁻¹ (KBr): 3447 (wb), 3360 (w), 3215 (m, -NH), 3062 (m, aromatic CH- str), 1662 (vs, C=O), 1641 (vs, C=N), 1606 (m), 1529 (s), 1253(s), 1024(s), 765 (s), 700 (s) (Fig. S2). ¹H NMR (400 MHz, CDCl₃, TMS): δ 11.50 (s, NH), 11.30 (s, 1H, NH), 8.60 (s, 1 H, azomethine), 8.5-6.9 (m, 12H, aromatic) (Fig. S3). ¹³C NMR (CDCl₃, δ ppm, TMS): 165.68, 161.53, 154.57, 148.22, 147.03, 139.51, 134.45, 133.69, 131.89, 130.22, 129.23, 127.91, 127.59, 127.02, 126.59, 124.43, 122.83, 120.14 (Fig. S4).

Synthesis of nickel complex (NiL₂, 2)

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A methanolic solution (10 mL) of Ni(NO₃)₂·3H₂O (0.120 g, 0.5 mmol) was added drop wise with stirring to the saturated solution of L (0.195 g, 0.5 mmol) in methanol (20 mL). The

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resulting mixture was continuously stirred for 1 hr. at room temperature and then filled the online online online online (h_{0} , h_{10} , $h_{$

Preparation of Stock solution for photo physical measurement

Receptor Lsolution was prepared initially at the concentration level of 1×10^{-3} M in 10 ml in methanol-*tris* HCl buffer medium (10mM, pH 7.2) solution (1:1 v/v), then diluted to the desired concentration. Also the stock solution of guest ions were prepared separately from their nitrate salts (except sulphate salt of Mn²⁺ and Fe²⁺ and potassium tetrachloroplatinate(II) salt for Pt²⁺) at a concentration of 1 x 10⁻³ M in 10 mL double-deionised water and further diluted to their desired concentration. After mixing L with each of the metal ions for a few seconds, absorption and fluorescence spectra were obtained at room temperature.

Computational details

The program package GAUSSIAN-09 Revision C.01 was employed for all calculations.³³ The gas phase geometries of the compound was fully optimized symmetry restrictions in singlet ground state with the gradient-corrected DFT level coupled with B3LYP.³⁴ Basis set LanL2DZ was used for the whole molecules L and NiL₂(2). The HOMOs and LUMOs of molecular ions were calculated with the same basis set and functional.

Cell imaging study

Human cervical cancer cell, HeLa was selected throughout the study. It was cultured in DMEM (Dulbecco's modified eagles medium) and supplemented with 10% Fetal Bovine Serum (FBS), and a 1% antibiotic mixture containing PSN (Gibco BRL) at 37°C in a humidified incubator with 5% CO₂. For cell imaging study the cells were seeded in a 35 mm culture dish with a seeding density of 3×10^5 cells per dish. Cells were grown to 80–90% confluence, harvested with 0.025% trypsin (Gibco BRL) and 0.52 mM EDTA (Gibco BRL) in phosphate-buffered saline (PBS) andallowed to re-equilibrate for 24h before any treatment. All experiments were conducted in DMEM containing 10% FBS and 1% PSN antibiotic. Cells were washed with PBS and then incubated with DMEM-containing 20 μ M chemosensor L [20 μ M in DMEM, DMSO:water = 1: 9 (v/v), pH 7.2 in PBS buffer] for 1h at 37°C. It was left for another incubation by adding 40 μ M of nickel nitrate salt and then fluorescence images were taken by a fluorescence microscope (Model: LEICA DMLS) with an objective lens of 20×magnification with excitation of 350-360 nm and emission 470 nm.

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Results and discussion

Synthesis and structure of L

In comparison to most of the N, O rich Schiff base receptor for Ni^{2+} ion chemosensor L has been prepared more easily and economically by coupling 3-amino-2-phenyl-4(3H)quinazolinone (1) with pyridine 2 carboxaldehyde with high yield (over 80%, Scheme 1) in a single pot.



Scheme 1 Synthetic procedure of the probe L.

We expected that presence of the peptide bonds and pyridine moiety would increase water solubility and coordination ability. The receptor **L** is highly soluble in 1:3 methanol-water mixture, but all the sensing studies have been carried out in methanol-*tris*HCl buffer medium (10 mM, pH 7.2) solution (1:1 v/v), as the solution is stable in this medium for more than two weeks. The chemical structure of **L** was confirmed by some solution phase measurements like ¹H-NMR, ESI-mass spectra and also with solid state measurements like IR spectroscopic studies, elemental analysis and single crystal XRD analysis.

Spectroscopic studies of L towards different metal ions

The metal selective response of chemosensor L was preliminary investigated by absorption spectral measurement in methanol-*tris*-HCl buffer (1:1 v/v, 10 mM, pH 7.2) solution at room temperature. As shown in Fig. 1a the free receptor L displays main absorption band at 310 nm due to S_0 - S_1 transition. Upon monitoring of 2 equiv. of different metal ions (40 μ M in each case) like Fe³⁺, Cu²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Mn²⁺, Cr³⁺, Ag⁺, Al³⁺ and Fe²⁺ to a fixed amount of L (20 μ M) solution, a red shifted absorption band appeared at 375 nm only in case of Ni²⁺ ion. As a result, colour of the solution changes from colourless to yellow. Except Cu²⁺ ion, the other tested metal ions can cause negligible change on absorption spectra of L. Although a pale yellow colorization is observed at that concentration level of Cu²⁺ion (40 μ M) but no sharp absorption spectra obtained suitable for colorimetric sensing study, even in addition of excessive amount. Considering physiological application, the selective responses of the receptor L towards Ni²⁺ ion was also investigated by competitive experiments in the same

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experimental condition. The co-existence of equivalent amount of common interfering cations of the second with Ni²⁺ ions (40 μ M Ni²⁺ with 40 μ M other metal ions) individually, cannot disturb the absorbance band around 375 nm. Although Pd²⁺ and Pt²⁺ ions belongs to the same group in the periodic table, they do not influence the absorption and emission spectra of L as well as L-Ni²⁺ complex. The L-Ni²⁺ peak remained almost constant even up to 5 fold higher concentration of background cations (100 μ M) with respect to receptor concentration, confirmed by neither generation of extra peak nor vanishing the original peak (Fig. 1b). Thus the receptor L exhibited specific selectivity towards Ni²⁺ion which might make it suitable for precise detection in complex mixtures.



Fig. 1 (a) Absorption spectra of L (20 μ M) changes in presence of 2 equiv. of different metal ions(40 μ M in each case). Inset: colour change of L(20 μ M)on addition of 2 equiv. of Ni²⁺(40 μ M) (b) Competitive experiment of L(20 μ M) towards Ni²⁺(40 μ M)in presence 2 equiv. other metal ions (40 μ M).

Next, to determine the sensitive response of L towards Ni²⁺ion, absorption titration experiment was performed in methanol-*tris*-HCl buffer (1:1 v/v, 10 mM, pH 7.2) mixture. In absence of guest, probe L showed two absorption bands at 286 nm and 310 nm corresponding to π - π * transition of multiple aromatic ring and n- π * transition of >C=O and >C=N group respectively. Upon gradual addition of Ni²⁺ ion to the solution of L (20 μ M), a systematic development of new absorption band appeared at 375 nm with a simultaneous decrease of the absorption band at 310 nm. The appearance of well-defined isosbestic point at 353 nm due to existence of dynamic equilibrium between L and L+Ni²⁺complex and 1.8 equiv. (36 μ M) of guest was enough to reach the plateau around 375 nm (Fig.2a).



Fig. 2(a) UV-vis titration of L (20 μ M) with Ni²⁺ (upto 40 μ M) in CH₃OH-*tris* buffer. Inset: Plot of intensity *vs.* analyte concentration. (b) Detection limits of L with Ni²⁺ by ratiometric measurements.

The ratiometric calibration curve was plotted from the titration experiments, by varying absorption intensity ratio (I_{375}/I_{310}) with increasing amount of Ni²⁺ion. Here, a sigmoidal curve was obtained with linearity range upto 20 μ M (Fig. 2b). The detection limits was found to 1.8 μ M using the formula, DL = 3 σ /m which belongs to lower level of the WHO guideline for drinking water and also a number of the previously reported Ni²⁺ sensors. The value of standard deviation σ is obtained from the plot of absorbance intensity versus [L] and slope m is obtained from ratiometric calibration curve (I_{375}/I_{310}) *vs* [Ni²⁺] plot.

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Host-guest binding stoichiometry can be determined from conventional Jobs plot analysis, where maximum intensity obtained at 0.7 mole fraction of L due to 2:1 host-guest complex formation(Fig. S7). Further, the 2:1 complex formation was supported by the mass spectra and elemental analysis of the isolated complex 2 (vide supra). The base peak obtained at m/z = 744.21, corresponds to $2L+Ni^{2+}-2H^+$ (Fig. S5). Considering 2:1 host-guest binding, the association constant towards Ni²⁺ was also calculated from Benesi-Hildebrand (B-H) linear fitting plot and the value obtained is $1.45 \times 10^3 \text{ M}^{-1/2}$ (Fig. 3a); which are highly in agreement to the previously reported L+Ni²⁺ complexes.



Fig. 3(a) Association constant from Benesi-Hildebrand plot; (b) pH effect of L and L–Ni²⁺ at 375 nm.

pH effect

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Host-guest binding affinity also dependant on the pH of the working media as it can modify the structure and coordination ability of pH sensitive groups (>C=O, >C=N, and -NH). To validate the pH dependency on complexation, a series of L solution (20 μ M) with pH range 3 to 12 have been prepared using HCl and NaOH in buffer medium and absorption studies were monitored in presence of Ni²⁺ion (40 μ M) (Fig. 3b). At very acidic condition (> pH 4) H⁺ ion acts as a competitor of metal binding, also a chance of imine >C=N bond breaking, which inhibits co-ordination ability and lowers the absorption intensity. The absorbance intensity of free receptor as well as its metal complex progressively increases to neutral-basic medium indicating that efficient electrostatic interaction present between deprotonated L and analyte ion. Thus the receptor L can effectively sense Ni²⁺ion with a wide pH range 5-12 also validate for physiological environment.

Reversibility and Logic gate

The reversibility of chemosensor L has been investigated by using common metal chelator disodium ethylene diamine tetra acetic acid (Na₂EDTA). The absorption bands around 375 nm along with yellow colour of host-guest complex immediately vanished with the appearance of original absorption band of L observed, when $L+Ni^{2+}$ complex treated with 1 equiv. strong chelator Na₂EDTA (Fig. 4a). Repeated addition of Ni²⁺ ion, the colour and spectral change almost recovered. The switching behaviour between L and $L+Ni^{2+}$ complexes were systematic even after 5 cycles with the alternative addition of chelator and analytes. Thus INHIBIT logic gate can be constructed by using Ni²⁺ and Na₂EDTA as a chemical input whereas output signal

based on absorbance intensity at 375 nm denoted by binary code "0" and "1"_{DOI}. The Very Addite Online behaviour and the corresponding truth table has been summarised in Fig. 4b.



Fig. 4 (a) Reversibility study of L-Ni²⁺ complexes with Na₂EDTA; (b) INHIBIT Logic gate and truth table.

Fluorescence experiment

The metal selective response of the receptor **L** in excited state was also investigated by fluorescence measurement (Fig.5a) in methanol-*tris*-HCl buffer (10 mM, pH 7.2) mixture. Due to excited energy loss of fluorophore by photo induced electron transfer (PET) and imine isomerisation via non-radiative pathway, the free receptor **L** showed weak emission band at 450 nm when exposed to UV light for excitation at 350 nm. Sequential addition of 2 equiv. of different metal ions (40 μ M in each case) like Fe³⁺, Cu²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Mn²⁺, Cr³⁺, Ag⁺, Al³⁺, Mn²⁺ and Fe²⁺ individually to the standard solution of **L** (20 μ M), only Ni²⁺ ion caused significant red shifted emission enhancement. Among the other metal ions, most of them showed either negligible change in emission band or quenching behaviour even upto 5 fold higher level of Ni²⁺concentration. The metal ion form tight complex via imine side and retard both PET and >C=N isomerisation, caused strong chelation enhancement fluorescence (CHEF) process.³⁵

Sensitivity has also been determined from fluorescence titration measurement. When the Ni²⁺ ion concentration gradually increased, the weak emission band of free receptor at 450 nm enhanced vertically with slightly red shifted in every step, and finally got saturated upon 2 equiv. of Ni²⁺ ion (40 μ M) (Fig. 5b). Simultaneously, the colour change of the solution also visible in UV lamp from light blue to greenish blue. The emission intensity at 465 nm showed linear relationship with amount of Ni²⁺ ion and the detection limit was calculated to **Dalton Transactions Accepted Manuscript**

1.18 μ M (Fig. S8). This value is slightly different from result obtained by absorption spectra difference on the difference in sensitivity of both the spectrometer. In the interfering effect, 2 equiv. Cu²⁺ ion can quench the emission intensity of L- Ni²⁺complex, but still measurable in the existence of Ni²⁺ion in the mixed solution (Fig. S9). The other metal ions including Pd²⁺ and Pt²⁺ do not exhibit any interference for the detection of Ni²⁺ion. Thus the chemosensor L is highly selective and sensitive fluorometric sensor for Ni²⁺.



Fig. 5(a) Fluorescence spectra of L (20 μ M) in presence of 2 equiv. different metal ions (40 μ M each) and (b) fluorescence titration of L (20 μ M) with Ni²⁺ (upto 40 μ M) in methanol-*tris* HCl buffer (1:1) mixture. Inset: colour change in UV lamp.

DFT studies on fluorescence properties

The probe L displays a simple approach for the selective detection of Ni²⁺ with enhancement of fluorescence along with red shift (15 nm), because of the CHEF (chelation-enhanced fluorescence) as well as ICT (internal charge transfer) processes after interaction with Ni²⁺. The energy gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of L and NiL₂ (2) are 3.96 eV and 2.68 eV respectively. The NiL₂ complex 2 compared to L are established the easy electronic transition and get the additional stability in 2 (Fig. 6). The contours of the electronic distribution in HOMO and LUMO states of these molecules suggested significant energy differences 1.28 eV between L and NiL₂ (2). Specifically, both HOMO and LUMO states of the nickel complex, 2 with comparing to L revealed that the electrons are more delocalized insight into the NiL₂ molecule than the L in agreement with the barrier of photo-induced electron transfer process, which may result in the enhancement of fluorescence through CHEF.³⁵



Fig. 6 Energy level diagram for the frontier MOs of L (left) and NiL₂ complex (2) (right).

¹H NMR studies

In order to investigate the probable binding interaction between host-guest complex, ¹H-NMR spectra of L and 2 have been taken in CDCl₃(Fig. S10). Presence of Ni²⁺, the azomethine proton of the free receptor L shifted towards downfield from $\delta = 8.62$ ppm (in case of L) to $\delta = 9.1$ ppm (for 2), confirmed that complexation occurred through imine site of L. Also the -NH proton of hydrazide moiety of L, at $\delta = 11.55$ ppm disappeared during Ni²⁺ binding, probably due to the dispositive charge of Ni²⁺was balanced by two deprotonation of L. Most of the aromatic protons in 2 were shifted towards downfield due to LMCT process. From the above mentioned facts the probable binding mechanism has been shown in Scheme 2 and confirmed by X-ray single crystal analysis of 2 (vide infra).

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Scheme 2 Proposed sensing mechanism of L.

X-ray single crystal structures of L and NiL₂(2)

To establish the NiL₂ complexation in solid phase, isolation in single crystal form is also necessary, as it is more acceptable in current research interest. Thus careful efforts have been taken to isolate the L-Ni²⁺complex in the form of single crystal at the experimental condition. Mixing of receptor-analyte in a 2:1 molar ratio, a block shaped red crystal was separated out, which was suitable for single crystal XRD analysis.

Geometry optimizations starting from the X-ray crystal structures of L and 2 (Fig. 7) led to a global minimum as stationary point. Selected geometry optimized parameters are listed in Table S1. The general drift observed in the crystal structure data is well reproduced. The calculated bond lengths as well as bond angles are in good agreement with the experimental crystal structures data (Table S1). In general, a little deviation of the calculated bond lengths of L and 2 deviate within range of $\pm 3\%$ from the respective observed crystal data, while the bond angles are vary within the range of $\pm 4\%$. Since the optimizations L and NiL₂ (2) are conceived under gas phase model.

The single crystals of the probe2-(benzamido)-N'-((pyridin-2-yl)methylene) benzohydrazide (L) and its Ni(II) complex (2) were grown by slow evaporation of the methanol solutions at room temperature. The crystallographic data of L andits nickel complex (2) are listed in Table 1. L crystallized as monoclinic C2/c space group whereas 2 crystallised as triclinic *P-1* space group. 2 crystallises with one molecule of H₂O. In this Ni(II) complex 2, Ni(II) ion adopted an octahedral coordination geometry comprising of two ligand L moieties

and one Ni(II) ion. The two cationic charges of Ni(II) ion were internally satisfied by $C_{0448D}^{\text{Online}}$ deprotonated hydrazide NH of two molecules of **L**. Moreover, coordination number 6 has been supplemented by coordinating to two molecules of L, leading to the formation of an innermetallic complex of the first order for the bivalent metal ion. The metal to donor bond distance values (Table S1) supports the low spin conformation for the NiL₂ complex **2**.

ORTEP view of **L** and **2** along with the atom numbering scheme is shown in Fig. 7. Close study of the crystal structure of **L** revealed no intra- or intermolecular π - π stacking in contrast to that observed in NiL₂ (**2**). The centroid-to-centroid separations between two pyridine rings of the ligands in **2** are 3.530 Å and 3.764 Å and get extra stability (Fig. 8).³⁶ Earlier this separation was recommended to be 3.80 Å to bring about considerable interaction. It is also notable that the offset situation of rings, the separation will be increased.³⁷



Fig.7 ORTEP diagrams of L (left) and NiL₂ (2) (right) showing the atom-labelling scheme and 30% thermal ellipsoids.



Fig. 8 View of NiL₂.H₂O (**2**.H₂O) showing, intramolecular π - π stacking interactions (magenta dotted line).

Table 1Crystallographic data and refinement parameters of the probe L and $2.\mathrm{H_2O}$

Identification Code	L	2 .H ₂ O
Empirical formula	C ₂₀ H ₁₆ N ₄ O ₂	C ₄₀ H ₃₂ N ₈ NiO ₅
Formula weight	344.38	763.44
Temperature/K	296.24	294.58
Crystal system	monoclinic	triclinic
Space group	C2/c	P-1
a/Å	11.2708(7)	10.445(7)
b/Å	12.1463(9)	13.227(9)
c/Å	25.4971(14)	14.693(10)
α/°	90	66.869(14)
β/°	98.351(2)	72.930(16)
γ/°	90	82.241(15)
Volume/Å ³	3453.5(4)	1784(2)
Z	8	2
$\rho_{calc}g/cm^3$	1.3246	1.4210
µ/mm ⁻¹	0.089	0.602
F(000)	1440.6	793.0
Radiation	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	5.04 to 54.24	4.44 to 57.18
Reflections collected	12225	54364
Independent reflections	$3794 (R_{int} = 0.0360,$	9010 ($R_{int} = 0.1036$,

	$R_{sigma} = 0.0402)$	$R_{sigma} = 0.076$ $R_{1: 10.1039/C9DT0244}$
Data/restraints/parameters	3794/0/235	9010/0/490
Goodness-of-fit on F ²	1.090	1.112
Final R indexes [I>= 2σ (I)]	$R_1 = 0.0481, wR_2 = 0.$	1129 $R_1 = 0.0648, wR_2 = 0.1693$
Final R indexes [all data]	$R_1 = 0.0756, wR_2 = 0.$	1344 $R_1 = 0.1149, wR_2 = 0.2129$
CCDC number	1917470	1917471

Application of the chemosensor L in real samples

As the industrial waste water was mixed with the normal water resources, the bio accumulation of Ni²⁺ ion is mainly initiated through water contamination. Thus efficiency of the chemosensor L towards waste water sample was checked. The accuracy of the measurement was estimated by spiking a known amount of standard Ni²⁺ solution and its recovery was calculated using calibration curve (intensity ratio *vs.* conc.) and Lambert-Beer law (Table 2). The recovered concentration Ni²⁺ ion by the receptor L are well fitted to spiked amount with satisfactory precision (RSD valueless than 2%) suggesting that the molecular probe L would be frequently used to detect Ni²⁺ ion in environmental samples.

Table 2 Determination of Ni²⁺ ion in water samples.

Metal Ion	Spiked amount (µM)	Recovered amount(µM)	Recovery %	RSD % value
	10	9.96	99.6	
Ni ²⁺	10	10.14	101.4	1.35
	10	10.23	102.3	

Smart Phone Based Colour Image

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To investigate rapid and convenient colorimetric determination of Ni²⁺ by the proposed receptor L, without other laboratory instruments, easy to handle smartphone based studies were also carried out. Thus various concentration levels of Ni²⁺contaminated sample was prepared in tap water and standard solution of probe L was added to the sample followed by the image of each sample was captured in flashlight by a smartphone (Fig. S11). Then the obtained images were analysed by an embedded application tool RGB grabber shunamicode in smartphone to get the value of red green and blue channel level and plotted with concentration of Ni²⁺ ion (Fig. S12). Among them, the blue to red (B/R) channel intensity ratio as a function of [Ni²⁺] ion showed most significant variation with good linearity (Fig. 9) obtained upto 25 μ M. The smartphone based detection limit was calculated to 1.52 μ M using same equation 3 σ /m. The

unknown concentration (X) also calculated using this standard curve (Fig. 9) by smarther Article Online image capture without any expensive and sophisticated instruments.



Fig. 9 Plot of the ratio of colour channel level.

Cell imaging study

To validate the affinity of receptor L towards Ni^{2+} ion inside the intracellular medium, cell imaging study has been performed by a fluorescence confocal microscope. Proper incubation of human cervical cancer cell, HeLa for 2h at 37^oC either by the receptor L or Ni²⁺ ion, individually showed only dark background, when continuously irradiated at 350 nm wave lengths. Under the similar condition, when the same cell pre-incubated by 40 μ M Ni²⁺ solution followed by 20 μ M probe L, a bluish green fluorescence image was observed (Fig. 10). These results clearly indicate that the receptor L can be applicable to detect Ni²⁺ ion inside the living system with good cell permeability and minimum interference from background.



Fig. 10 Fluorescence microscopy images of the Hela cells after incubation for 2 h: (a) cells + probe L (20 μ M), (b) cells + Ni²⁺(40 μ M) and (c) cells +probe L (20 μ M) + Ni²⁺ (40 μ M).

Comparison of L with other reported Schiff-base chemosensors

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In continuation of our ongoing effort for providing useful analytical techniques to monitor the increasing number of analytes of environmental relevance as quickly and as cheaply as possible and with the highest order of sensitivity, the present paper describes the synthesis of a new hydrazide based optical probe, (L), which was validated for detection and quantification of Ni^{2+} ions in real samples. The probe L is readily soluble in aqueous methanol (1:1 CH₃OH-H₂O) which produces a detectable color change in presence of Ni²⁺ion and has excitation/emission wavelengths very close to the visible region.

1 1	icaulty soluble	in aqueous	methano	ы (1.1 Сп ₃ Оп		
H_2O) which produces a detectable co	olor change in	n presence	of Ni	²⁺ ion and ha	s	
excitation/emission wavelengths very close to the visible region.						
To the best of our knowledge, few previously reported Schiff base chemosensors for						
Ni ²⁺ ion were also available. Here, the synthesized probe L was compared with those reported						
chemosensors (Table 3). Although most of the other Schiff-base chemosensors showed						
somewhat lower detection limit value compared to this proposed sensor, but there were some						
limitations like: only colorimetric respond	limitations like: only colorimetric responds, low pH range and only solution based detection					
without isolation of single crystal of the host-guest complex etc. The important features of the						
proposed chemosensor L are: not complic	ated synthetic p	procedure, h	igh sensi	tivity, wide pl	H d	
range (5-12), reversibility in binding to the analyzed cations, high accuracy and precision,						
isolation of host-guest complex in single crystal form, application in contaminated water						
sample, living cell imaging and smartphone based analysis. Thus the proposed chemoreceptor						
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gives a new arena in the field of Schiff base Table 3 Comparison of L with other report	e chemosensor. ed Schiff-base c	hemosensor	s	cnemorecepto		
gives a new arena in the field of Schiff base Table 3 Comparison of L with other report Chemical structure of the probe	e chemosensor. ed Schiff-base c Sensing method	hemosensor Binding Constant	s LOD	I cnemorecepto Isolation in form of single crystal	Ref.	
gives a new arena in the field of Schiff base Table 3 Comparison of L with other report Chemical structure of the probe $I = \frac{1}{2} \int_{0}^{N} \int_{0}^{1} \int_{0}^{N} \int_{0}$	e chemosensor. ed Schiff-base c Sensing method Colorimetric	hemosensor Binding Constant 2.34 x 10 ⁴ M ⁻¹	s LOD 0.5 μM	I cnemorecepto Isolation in form of single crystal No	Ref. 000	

Сғ _{зн}	Colorimetric/			View Article DOI: 10.1039/C9DT0	e Onigne 12449D
	Fluoremetric				
$ \underbrace{ \underbrace{ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	Colorimetric		1.47 μΜ	No	41
	Colorimetric	1.29 x 10 ⁵ M ⁻¹	0.1 μΜ		42 0000
	Colorimetric/ Fluoremetric			Red crystal	43
	Colorimetric	4.01 x 10 ⁶ M ⁻¹	0.36 μM	No	44 DOC Stions
	Colorimetric			Red crystal	Present work

Conclusions

In summary, we have successfully designed and synthesized a quinazolinone derived Schiff base chemosensor L for fluorescent colorimetric detection of Ni²⁺with high selectivity and sensitivity over other competitive ions in aqueous medium. A 2:1 stoichiometry between L and Ni²⁺were supported by Job-plot, ESI-mass spectral analysis and DFT studies. The single crystal of the probe L and its nickel complex 2 were also isolated. The detection limits reached up to 1.8 μ M and 1.18 μ M respectively by colorimetric and fluoremetric measurements, which were **Ualt**

far lower than those recommended by the WHO guidelines for drinking water. The reversible Online response to Ni²⁺ion was established by the Na₂EDTA experiments. Moreover, L could operate in a wide range of pH and can be successfully applied for detection and quantification of Ni²⁺ in environmental samples, smart phone based analysis, living cell imaging and for building INHIBIT type of logic gates. On the basis of these results, we believe that receptor L will be a remarkable supplement in the sphere of simple Schiff base sensor for Ni²⁺ion.

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Supporting Information Summary

CCDC 1917470 and1917471contain the supplementary crystallographic data for the probe Land the complex **1**. The data can be obtained free of charge from the Cambridge Crytallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; Fax: (+44)1223-336-033; or email: <u>deposit@ccdc.cam.ac.uk</u>: Supplementary data containing Tables S1 and Fig. S1-S12 of this article can be found online at.....

Conflicts of interest

Authors declare no conflicts of interest.

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Graphical Abstract

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Hydrazide based novel selective and sensitive optical chemosensor for the detection of Ni²⁺: Applications in living cell imaging, molecular logic gate and smart phone

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A novel flexible hydrazide based Schiff base chemo-receptor 2-(benzamido)-N'-((pyridin-2-yl)methylene)benzohydrazide (L) has been designed, which showed colorimetric and fluorometric "*turn on*" response in presence of only Ni^{2+} . The chemosensor L can be applied to the recovery of contaminated water samples, building of molecular logic gate, smart phone and living cell imaging.

