

A novel water-soluble highly selective “switch-on” ionic liquid-based fluorescent chemi-sensor for Ca(II)



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ABSTRACT

A novel chemi-sensor involve new bis-ionic Schiff base sensor (BISBS), *N,N'*-bis-[5-((2,4-lutidiniumchloride)methylene)-3-methoxysalicylidene]-*R,R*-1,2-cyclohexanedimine, has been synthesized and characterized. BISBS chemi-sensor was designed based on internal charge transfer (ICT) fluorescence mechanism. This new water soluble chemi-sensor provides great selectivity fluorescence detection for Ca(II) ions in an important physiological pH range. Moreover, the interaction of Ca(II) with the deprotonated BISBS to produce a metal-ligand complex with a ratio of (1: 1) accompanying with an enhancement in the intensity of emission band located at 502 nm. Fluorescence switching-on during the chemical interaction between BISBS and Ca(II) ions is very easily noticed with naked eye, but other metal cations such as alkali, alkaline earth and transition metal don't give any fluorescence changes. The novel developed BISBS sensor successively offers low limit of detection (LOD) 1.5 nM and fast tracing of Ca(II) in the physiological pH 7.6. Thus BISBS may provide a novel auspicious methodology for detection calcium cations in the environmental and biological samples.

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1. Introduction

Development and designing of molecular sensing systems, exhibiting significant spectrophotometric or fluorescence changes upon chelating analytes has attracted much attention. Design of sensitive and selective molecular systems for tracing different types of metal ions particularly Ca(II) ion, which takes a crucial systematically role in diverse bio-systems, has been receiving much interest [1]. Moreover, the Ca(II) ions exhibited potentially high importance in many fields including food technology, environment [2] and medicine [3]. Many molecular probes were designed for detection and measuring Ca(II) ions based on different molecular type such as organic dye [4], protein [5] and nanoparticles [6]. Chemi-sensor probes are predominantly attractive because they allow simple naked-eye detection of Ca(II) ion. Thus, different types of colorimetric probes have been synthesized in order to detect calcium ions, but most of these probes suffer from interfering detection due to the presence of other metal ions such as zinc metal ions [7].

Concurrent detection of the specified analyte based on the change of fluorescence property is frequently the most utilized method that accompanied by decreasing in the fluorescence intensity (quenching) “Turn off” mechanism or enhancing the fluorescence intensity of the sensor molecules in the presence of analyte or metal ions “Turn on” mechanism [8]. However, the change in the fluorescence intensities of these chemi-sensor molecules could be attributed to many factors as system temperature, environmental pH, photobleaching and polarity of solvent. Recently, the developed optical sensors for Ca(II) ions were developed mainly by Ca(II) ion replacement for a hydrogen proton of the pH-sensitive dye or chelating of Ca(II) ion sensitive dye with neutral ion carriers [9]. Moreover, the fluorescent Ca(II) chemi-sensor becomes significant technique for screening Ca(II) ions.

The extreme advantage properties of fluorescence turn-on sensing mechanism with respect to turn-off mechanism are the low detection limit of the specified analyte and the relative dark fluorescence background of the chemi-sensor [10]. These factors decrease the probability of pseudo signal and increase both sensitivity and selectivity of chemi-sensor. For turn-on and turn-off mechanisms, ICT internal charge transfer has been extensively utilized in order to interpret the fluorescence behavior of various sensors [11]. Moreover, the visual sensing based naked eyes would provide strong tool with high sensitivity and selectivity towards the specified analyte. Design and synthesis of Schiff base fluorescence chemi-sensor based ICT mechanism has a great attention

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and has been used widely as DNA cleavage [12] and nonlinear optical materials [13].

The relentless ease of preparation of Schiff-base derivatives anchoring fluorescent moieties along with their amazing photophysical properties make them promising and attractive candidates for building up of novel chemi-sensors for fluorescence detection of numerous types of metal cations [14]. Noteworthy, the isomerization of azomethine (H—C=N) fragment belongs to a Schiff-base is reasonable for the decay mechanism of the excited-states of Schiff-bases with an un-bridged C=N skeleton that led to a nonfluorescence profile [15]. Hence, enhancement or restriction of this isomerization *via* metal ions binding will play a crucial role in fluctuation of fluorescence intensity accompanied these compounds. Additionally, Schiff-bases have been recognized as chemi-sensors for fluorometric and colorimetric detection of Hg²⁺ and Ca²⁺ cations (20 μM and 0.3 μM, respectively). The interference of other cations is completely ineffective [16].

The amazing physical and chemical properties of ionic liquids (ILs) [17] along with their extensive potential in many applications such as COSMO-RS analysis [18], azeotropic separation process [19], microbalance sensors and designer solvent in chemical synthesis besides electrochemistry [20,21] make them attract attention of numerous analytical researchers. Recently, they have used to design ionic liquid-based fluorescent nanosensors [22] and Schiff-base chemi-sensor [16b]. Inspired with the aforementioned facts, we intended herein to develop a new, simple ultrasensitive and selective chemi-sensor for Ca(II) with efficient dual-solubility in aqueous and organic solvents based on a Schiff-base probe bearing ionic liquid compartments.

2. Experimental

2.1. Reagents and materials

See supplementary content.

2.2. Instrumentation

See supplementary content.

2.3. Synthesis of 1-(5-formyl-vanillyl)-2,4-lutidinium chloride (MeO)sal(Lut⁺Cl⁻) (2)

To a vigorously stirred solution of 2,4-lutidine (0.52 g, 4.87 mmol) in anhydrous benzene (25 mL), the solution of 5-chloromethyl-3-methoxysalicylaldehyde [16b] (1.96 g, 9.8 mmol) in anhydrous benzene (50 mL) was added, drop-wise over 30 min, under nitrogen atmosphere at room temperature. The resulting solution was further stirred at 80 °C for 24 h. After cooling to room temperature, the isolated product was washed intensively with 5 × 10 mL anhydrous benzene, several times with ether (5 × 10 mL), to remove the unreacted materials, and finally dried under high vacuum to give the lutidinium salt (2) which used for the following preparation without further purification. Compound 2 was obtained as a faint yellow solid (2.68 g, 89%), mp = 86–87 °C. FTIR (KBr, cm⁻¹): 3224 (m, br, ν_(O-H)), 3031 (m, sh, ν_(C-H)), Ar), 2982 (m, sh, ν_(C-H), Aliph.), 1686 (vs, sh, ν_(C=O)), 1643 (vs, sh, ν_(C=O)), 1569, 1496, 1390 (s, sh, ν_(C=C_{Ar}+C=N_{Py})), 1286 (s, sh, ν_(Ar-O)), 1144 (s, sh, ν_{(H-C=C+H-C=N)_{bend}}, Py). ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm) (Fig. S1): 10.32 (s, 1H), 8.06 (s, 1H), 7.85–7.72 (m, 2H), 7.31–7.15 (m, 3H), 5.39 (s, 2H), 3.89 (s, 3H), 3.79 (s, 3H), 2.67 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm) (Fig. S2): 191.11, 151.37, 149.30, 144.85, 137.70, 129.25, 128.56, 125.85, 125.67, 122.95, 122.67, 121.43, 119.44, 56.34, 50.51, 35.16 and 21.41. ESI MS: in positive mode peaks at *m/z* = 272.1 ([C₁₆H₁₈NO₃]⁺, M—Cl) a.m.u.

2.4. Synthesis of (1*R*,2*R*)-1,2-diammoniumcyclohexane *L*-tartrate (3)

It was prepared according to the method reported by Elshaarawy et al. [23] with slight modifications, in brief, at 70 °C, *rac-trans*-1,2-diaminocyclohexane (6 mL, 50.0 mmol) was added dropwise to a solution of *L*-(+)-tartaric acid (7.5 g, 50.0 mmol) in distilled water (20 mL). Then the reaction temperature was gradually increased until 90 °C in order to keep the mixture solubilized. The resulting slurry was stirred at the same temperature for a further 2 h, and then the isotropic solution was allowed to spontaneously cool down to RT and then kept at 4 °C overnight. The resulting precipitate was collected by vacuum filtration and washed with 5 °C distilled water (2 × 25 mL) and then methanol (5 × 15 mL). The crude product, 3, was then recrystallized by dissolving the compound in distilled water at 90 °C and leaving to cool to RT overnight. The recrystallization process was repeated twice. The purified product was collected by vacuum filtration and dried under reduced pressure. ¹H NMR (200 MHz, D₂O) δ (ppm): 4.64 (d, *J* = 7.1 Hz, 2H), 4.15 (td, *J* = 10.8, 3.9 Hz, 1H), 3.98 (td, *J* = 10.6, 4.1 Hz, 1H), 2.71 (3, 6H), 2.38–2.13 (m, 4H), 1.67–1.89 (m, 4H).

2.5. Synthesis of (1*R*,2*R*)-1,2-diaminocyclohexane (*R,R*-DACH) (4)

A NaCl-saturated solution of NaOH (1.91 g, 47.8 mmol) in H₂O (25 mL) was added dropwise to a solution of diammoniumcyclohexane *L*-tartrate 3 (3.17 g, 12.0 mmol) in H₂O (25 mL). After stirring for 30 min at ambient temperature the mixture was extracted with CHCl₃ (5 × 50 mL). The combined extract was dried over anhydrous MgSO₄. The solvent was evaporated under reduced pressure and the residue submitted to recrystallization from petroleum ether (40–60) to yield the desired product, *R,R*-1,2-diaminocyclohexane (4) as white crystals which stored at –20 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 4.99 (s, br, 4H), 2.93 (td, *J* = 10.7, 4.0 Hz, 2H), 2.01–1.88 (m, 4H), 1.31–1.17 (m, 4H).

2.6. Synthesis of new bis-ionic Schiff base sensor (BISBS) *N,N'*-bis-[5-((2,4-lutidinium chloride)methylene)-3-methoxysalicylidene]-*R,R*-1,2-cyclohexanedi-imine (5)

Into a 100 mL Schlenk flask, a methanolic solution (10 mL) of *R,R*-DACH (0.23 g, 2.0 mmol) was added dropwise to a solution (20 mL) of methoxysalicylaldehyde-2,4-lutidiniumchloride (MeO)sal(Lut⁺Cl⁻) 2 (1.23 g, 4.0 mmol) in methanol under N₂ atmosphere. The reaction mixture was refluxed with stirring under N₂ for 6 h. Then the solvent was partially removed in rotatory evaporator, and a yellow product of chemi-sensor (5) was departure from the solution by the addition of ethyl acetate along with keeping in the refrigerator overnight. Then, the solvent was decanted off and the isolated crude product was sonicated for 15 min in Et₂O (3 × 25 mL). Et₂O was also decanted off and the residual solid was washed intensively with MeOH/Et₂O mixture (1:2) to remove unreacted materials and then re-dissolved in MeOH. EtOAc was added slowly (during ~15 min) to precipitate the product 5 which was collected by filtration and dried under vacuum. New chemi-sensor, 5, was obtained as a yellow-orange powder, (2.30 g, 83%), mp = 181–182 °C. FTIR (KBr, cm⁻¹): 3436 (m, br, ν_(O-H)), 3135 (w, br, ν_{asym(C-H)}, Py and Ar), 3074 (m, br, ν_{sym(C-H)}, Py and Ar), 1634 (vs, sh, ν_{(C=N)_{Azomethine}}), 1558, 1460, 1377 (s, sh, ν_{(C=C_{Ar}+C-H)_{bend}}), 1270 (m, sh, ν_(Ar-O)), 751 (s, sh, ν_{(H-C=N)_{bend}}, Py). ¹H NMR (200 MHz, CDCl₃) δ (ppm) (Fig. S3): 10.58 (s, 1H), 10.53 (s, 1H), 8.45 (d, *J* = 7.4 Hz, 2H), 8.24 (s, 2H), 7.61 (s, 2H), 7.38 (d, *J* = 1.8 Hz, 2H), 7.30 (s, 2H), 6.97 (s, 2H), 5.43 (s, 4H), 4.26–4.16 (m, 2H), 4.05 (s, 6H), 3.79 (s, 6H), 2.42 (s, 6H), 2.05–1.88 (m, 4H), 1.34–1.19 (m, 4H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm) (Fig. S4): 171.5, 165.26, 152.53, 148.74, 144.74, 124.01, 123.14, 122.88, 121.26, 118.02, 114.92, 70.96, 56.22, 50.70, 39.72, 35.14, 35.10, 32.90 and 24.03. ESI-MS (*m/z*, a.m.u.): calcd for C₃₈H₄₆ClN₄O₄ and C₃₈H₄₆N₄O₄: 658.3 [M—Cl]⁺ and 622.8

$[M-2Cl]^{2+}$; found: 658.2 and 622.7, respectively. Anal. Calcd. for $C_{38}H_{46}Cl_2N_4O_4$ ($M = 693.70$ g/mol): C, 65.79; H, 6.68; N, 8.08; found: C, 65.68; H, 6.71; N, 7.99.

2.7. Fluorescence and UV-vis measurements

Alkali, alkaline and transition metal stock solutions were prepared. The fluorescence titration of the new BISBS free ligand was achieved in aqueous solution $0.8 \mu\text{M}$ concentration, 20 mM HEPES buffer and at pH 7.6. The UV-vis spectra measurements of BISBS and metal complexes were recorded in an aqueous solution with $0.4 \mu\text{M}$. During Absorption titration, the net volume of BISBS and Ca(II) should be kept 2.0 mL cause the volume of Ca(II) could be neglected with respect to BISBS.

3. Results and discussion

3.1. Synthesis protocol of new sensor

The synthesis of *N,N*-bis-(3-methoxysalicylidene)-*R,R*-1,2-cyclohexanedimine anchoring lutidinium chloride terminals (**5**), a new chemi-sensor, is depicted in Scheme 1. At the outset a new lutidinium ionic liquid (MeO)sal(Lut⁺ Cl⁻) (**2**) was prepared, in a highly pure state, starting from *o*-vanillin via a combined chloromethylation-quaternization protocol as shown in Scheme 1. Thereafter, a simple Schiff base condensation between new lutidinium ionic liquid and a chiral *R,R*-DACH was the followed strategy for preparation our bis-ionic Schiff base fluorescent chemi-sensor (BISBS) (**5**). This chemi-sensor was isolated in high yield and structurally characterized using elemental analysis and spectral methods (FTIR, UV-vis, NMR (¹H, ¹³C), ESI-MS).

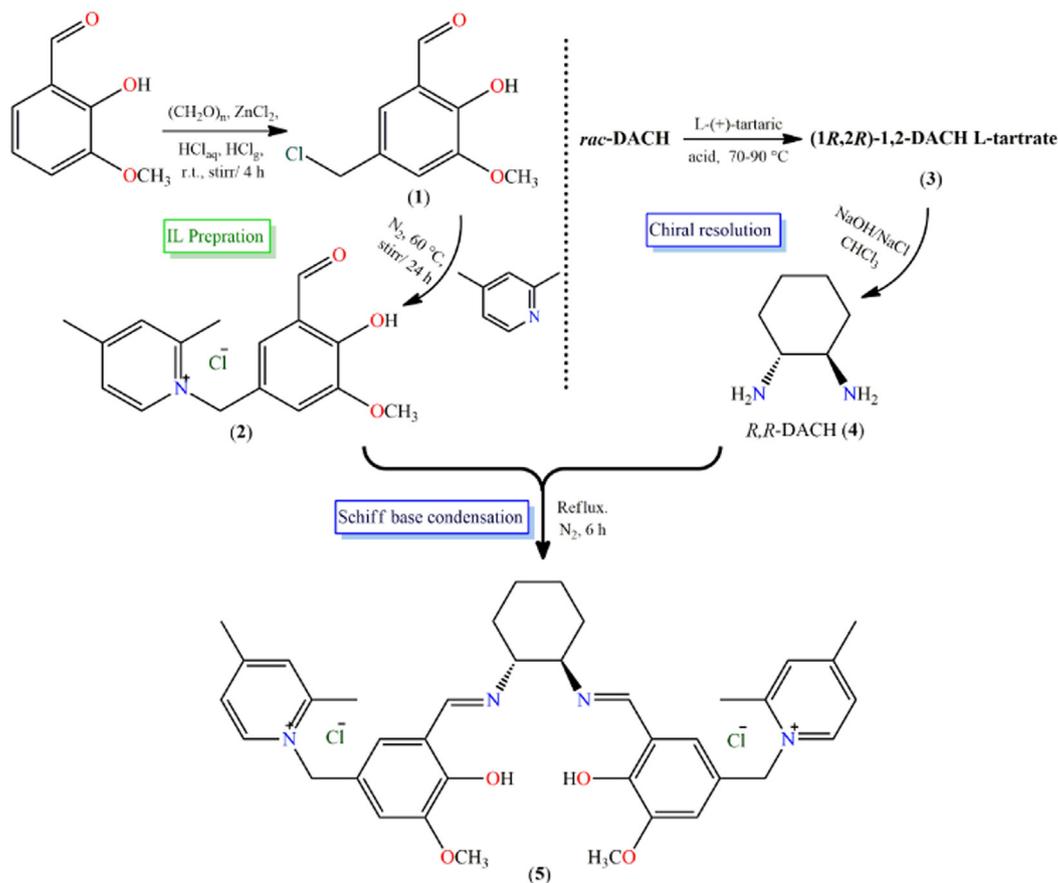
3.2. Characterization of BISBS

Notable that the molten salts of melting point (mp) below $100 \text{ }^\circ\text{C}$ are recognized as ionic liquids (ILs). Accordingly, our new sensor could not be classified as IL, however, it is considered as a ionic molten salt as its mp $> 100 \text{ }^\circ\text{C}$ ($181\text{--}182 \text{ }^\circ\text{C}$) anchoring vanillyl-2,4-lutidinium chloride ionic liquid of mp ($86\text{--}87 \text{ }^\circ\text{C}$).

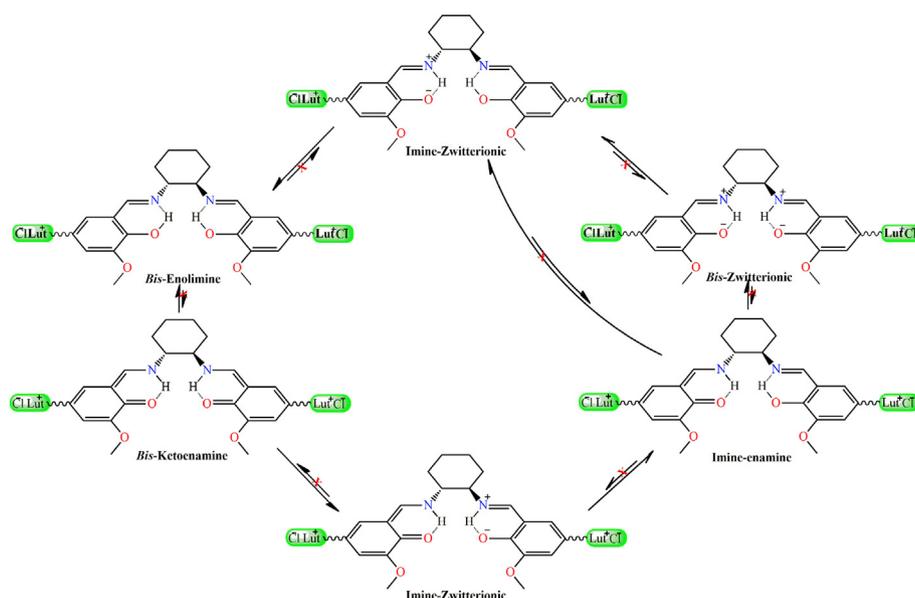
The obtained data from CHN microanalytical analysis for new bis-ionic Schiff base sensor (BISBS) was in good agreement with the suggested molecular formula for BISBS (see the Experimental section). Moreover a positive mode electrospray ionization mass spectra (ESI-MS) of new sensor (**5**) exhibited two characteristic peaks at $m/z = 658.2$ and 622.7 a.m.u. assignable to singly- and doubly-charged cations produced via departure of chloride ions, $[M-Cl]^{+}$ and $[M-2Cl]^{2+}$, respectively.

FTIR offers primary spectral markers for proofing the successful formation of new sensor (**5**) as revealed from its FTIR signatures such as: (i) an intense broad hump centered at 3436 cm^{-1} along with a maxima at 1270 cm^{-1} can assigned as a phenolic O—H fragment belongs to an intramolecular H-bonding environment (O—H \cdots N). (ii) Displacement of azomethine (—HC=N—) stretch to a lower-energy region, 1634 cm^{-1} , as compared with that reported for free azomethine fragment ($1657 \pm 3 \text{ cm}^{-1}$) [24,25] provides a further evidence for the participation of azomethinic group in a H-bonded six-membered chelating ring (see Scheme 2). (iii) Anchoring of bis-Schiff base to the lutidinium compartments can be detected from the observation of three main IR peaks at 1597 , 1158 and 751 cm^{-1} , characteristic for pyridinium moiety.

(iv) Noteworthy, missing of any vibrational mode, around 1700 cm^{-1} , attributable for C=O stretching vibration, offers a plausible evidence for the predominant population of O-protonated



Scheme 1. Schematic diagram for the synthesis of lutidinium ionic liquid (MeO)sal(Lut⁺ Cl⁻) (**2**) and bis-ionic Schiff base fluorescent chemi-sensor (BISBS) (**5**).



Scheme 2. Possible tautomeric forms along with intramolecular hydrogen-bonding in BISBS (5).

bis-enolimine tautomer in the keto–enol tautomeric equilibrium of solid BISBS (*cf.* Scheme 2).

^1H NMR provides the second and strong spectral markers confirming the successful formation of new sensor (5) and the predominance of bis-enolimine tautomeric form with little contribution from imine-zwitterionic form in the deuterated solution of BISBS. This can be easily concluded from the observed ^1H NMR spectral peculiarities for BISBS including: (i) two characteristic singlets at 10.58 and 10.53 ppm attributable for the resonance of phenolic O—H, typical for bis-enolimine tautomer. (ii) A doublet at 8.98 ppm ($J = 7.1$ Hz) which could be ascribed to resonance of iminic proton, typical for an ethylenic proton of zwitterionic (phenolate-iminium) fragment. This splitting pattern is in full agreement with our former suggestion regarding the relative tautomer, imine/enamine, population in deuterated solution of BISBS (*cf.* Scheme 2) [26].

3.3. The optical measurements of the new sensor

The optical properties of the new synthesized optical sensor BISBS was detected in solution (Fig. 1). The optical sensor exhibits an emission band located at 502 nm under excitation of 380 nm and the UV–visible

absorption spectrum shows three mean peaks located at 250, 325, 380 nm.

3.4. pH dependence of the fluorescence intensity of ionic Schiff-base

The detection of metal ions in solution using the fluorescence change of chemi-sensor mainly produced with transfer of hydrogen proton, this interaction accompanied by alteration the pH of the environment solution. Therefore, the new designed BISBS was tested utilizing fluorometric titration by alteration pH of the aqueous medium (see Fig. 2). In low range of pH value, the fluorescence intensity of the BISBS has a significant decrement within range (1 to 5.6). In contrast, the fluorescence intensity has a constant value over a wide range of pH (5.6 to 12). The fluorescence enhancement explanation of the chemi-sensor is mainly attributed to the (ICT) process [27]. The high affinity of BISBS towards water solubility led us to carry out all series of experiments in physiological pH 7.6 buffered systems with 20 mM HEPES buffer.

3.5. Fluorescence property of ionic liquid-based Schiff-base sensor (BISBS)

Fluorescence intensity of BISBS and its fluorometric titration with Ca(II) ion solutions of different concentrations were detected in

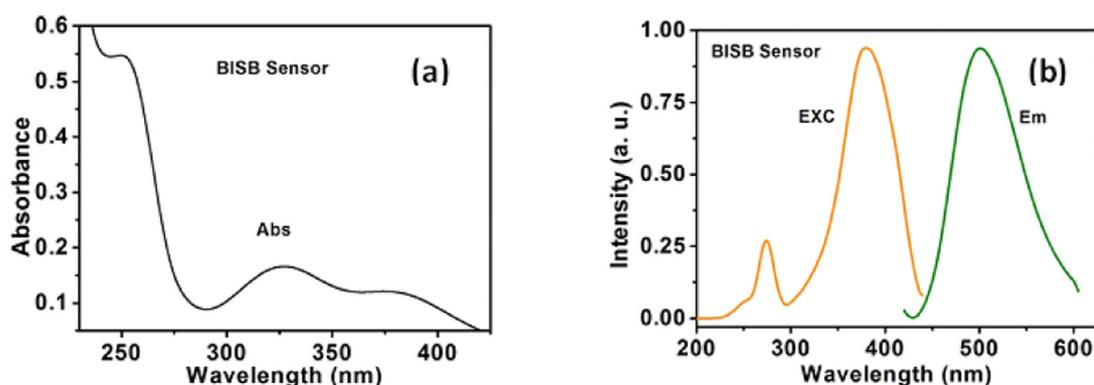


Fig. 1. (a) UV–vis spectrum and (b) excitation, and emission spectra under 380 nm of the BISBS sensor at pH 7.6, 20 mM HEPES buffer.

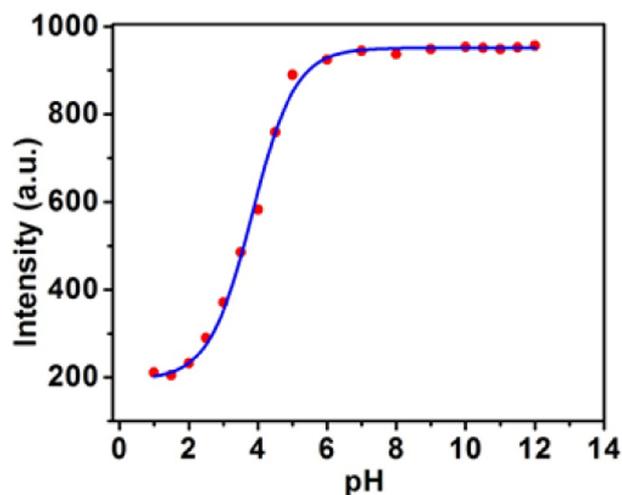


Fig. 2. Influence of the pH-value on emission intensity of the BISBS. Under excitation 380 nm and $[BISBS] = 0.8 \mu M$.

buffered aqueous medium (20 mM HEPES buffer at pH 7.6) as shown in Fig. 3. The fluorescence emission of the free BISBS shows a broad band with feeble emission peak centered at 502 nm. The free BISBS in buffered aqueous medium was titrated with regular adding Ca(II) cations. During the titration, a significant band enhancement centered at 502 nm was recorded. Simultaneous enhancement of the main BISBS emission peak revealed the formation of Ca(II)-BISBS complex. The signal intensity is constant with further addition of Ca(II) ions with respect to BISBS concentration. This indicates that the stoichiometric of metal to ligand interaction is (1:1). The fluorescence enhancing the behavior of the emission intensity of BISBS is referred to the ICT mechanism based the receptor Ca(II) and the donor BISBS. The response of the BISBS sensor to the Ca(II) cations was measured in buffered solution medium with concentration limits of 1–1.6 μM . The limit of detection (LOD) was calculated as 1.5 nM with a precision of $\pm 1\%$. Hence, the precious value of LOD for BISBS enables to detect Ca(II) ions with high sensitivity and selectivity. Besides, in future we can use this chemi-sensor can be used efficiently in determination of Ca(II) in environmental samples.

Interestingly enough, the lutidinium ionic liquid terminals act as a multiple-functions segments where the amphiphilic nature of these moieties enhances the dual-solubility of the new sensor in both aqueous and organic solvents allowing the sensing of Ca(II) in biological samples. Moreover, the positive charges of two lutidinium moieties form assemblies with nitrate ions in aqueous solution which promote the ICT from the anionic nitrate to cationic and consequently, fluorescence intensity and sensitivity for Ca(II) ion is

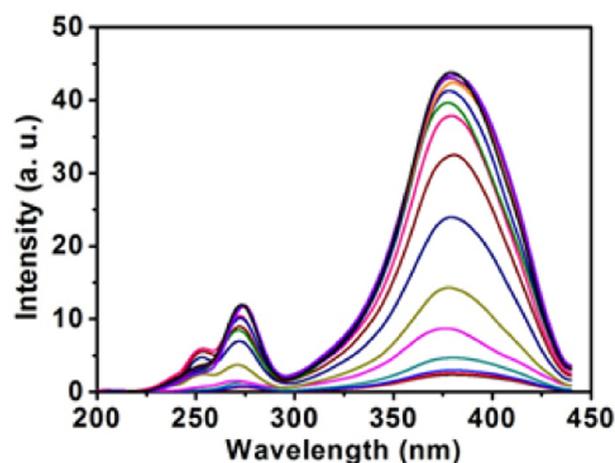


Fig. 4. The excitation spectra of the new BISBS sensor in free form and after titration with Ca(II) ions using Ca(II) within range (0–1.6 μM).

significantly increased, thereby, its limit of detection (LOD) by new sensor decreased.

The excitation spectra of the (0.8 μM) free ligand BISBS sensor and BISBS metal complex in the range of Ca(II) cations (0–1.6 μM) were determined with optimum aqueous conditions using 20 mM HEPES buffer, pH 7.6. Fig. 4 exhibits the excitation spectra of the free BISBS ligand and the Ca(II) complex with a considerable enhancement of the excitation peak at 380 nm which could be referred to the formation of the Ca(II) metal complex. When the metal-BISBS ratio reaches 1:1 during titration with Ca(II) cations, the excitation intensity of BISBS ligand has a constant value.

UV-vis spectra of the BISBS are measured under same optimum aqueous conditions. The absorption spectra show three main peaks centered at 250, 325 and 380 nm, the two characterized peaks centered at 250 and 325 nm were disappeared. On the other hand, the peak centered 380 nm was enhanced gradually adding Ca(II) ions to aqueous solution of free BISBS chemi-sensor. Further, the significant changes in the UV-visible spectra of the BISBS during titration with Ca(II) ions proved the formation of Ca(II)-BISBS complex as shown in Fig. 5. Furthermore, UV-visible spectra show two isopestic points at which BISBS absorbance doesn't alter through titration process. The absorption spectra turn constant without any alteration when the BISBS to Ca(II) ions ratio is becoming (1:1). Thus, this absorbance behavior proves that the stoichiometry of metal-ligand ratio of the formed complex is 1:1. Additionally, The red shift of the absorbance spectra is due to the change of the ICT process which involves the interaction of Ca(II) receptor and BISBS as a donor species.

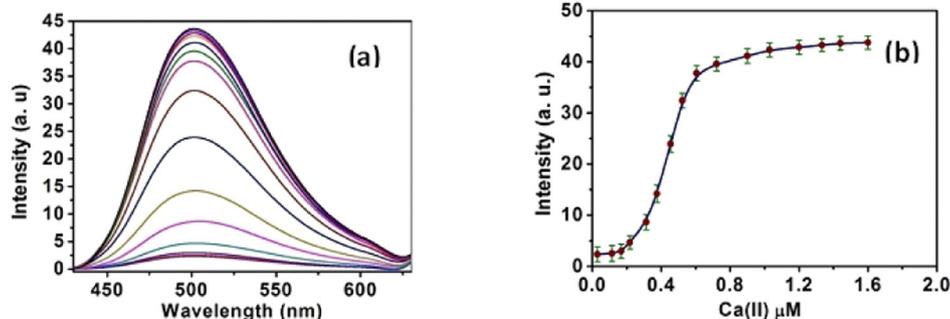


Fig. 3. (a) The effect of $[Ca(II)]$ from 0 to 1.6 μM , on the emission intensity of BISBS sensor. λ_{exc} was 380 nm, and λ_{em} were at 502 nm. $[BSB-IL]$ was 0.8 μM and (b) calibration curve I_{502} as a function of $[Ca(II)]$.

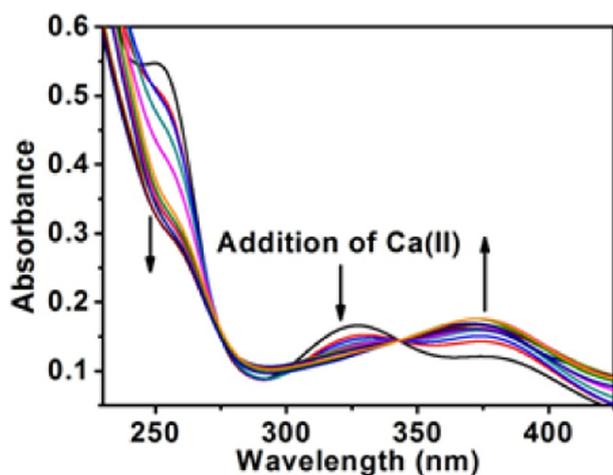


Fig. 5. UV-vis spectra of BISBS free ligand and Ca(II)-BISBS complexes (free ligand, formation of Ca complexes and saturation).

The influence of the other metal ions on the fluorescence intensity of the new BISBS sensor was studied under optimized conditions. Different types of alkali, alkaline-earth and transition metal ions were utilized. Particularly, most of the metal ions show a small alteration or quenching effects on the fluorescence intensity of the free BISBS ligand in aqueous medium containing of 20 μM HEPES buffer, pH 7.6. The BISBS was excited under 380 nm. Fig. 6 represents the effect of different metal ions on the fluorescence maxima of the new chemi-sensor. The concentrations of the free ligand and the specified metal ions were M 0.8 μM and 1.6 μM respectively. The relation gives precious prove about the high response of the BISBS to the Ca(II).

Moreover, the sensitivity and selectivity of the free BISBS chemi-sensor to Ca(II) were examined in presence of other significant metal ions. Fig. 7 show a great potential of the BISBS ligands towards Ca(II) ions with a highly chelating interaction. The sensing process of 0.8 μM BISBS with 1.6 μM concentration Ca(II) was carried out in presence of different cations at 1.6 μM , and within mix with different metal cations, respectively. The solution condition proceeded at pH 7.6 and 20 μM HEPES buffer. Data show that there is no influence or significant alteration of the fluorescence property of BISBS with Ca(II) with existence or absence of different metal ions.

Selective binding of Ca(II) compared to other metal ions could be attributed to: (i) the compatibility between the ionic size of M^{2+} and

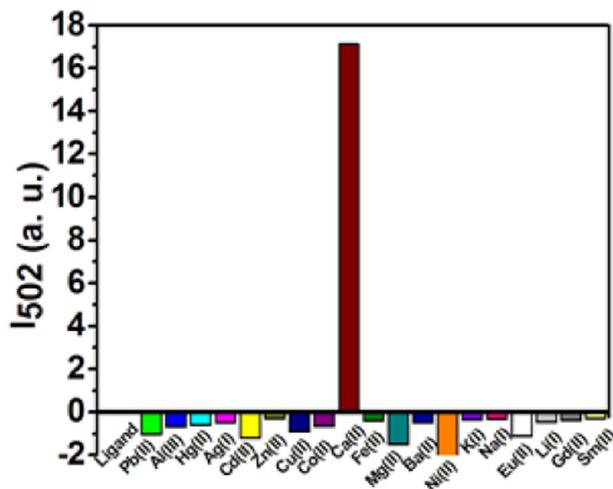


Fig. 6. The influence of different metal ions on the fluorescence of the BISBS new sensor.

the dimension of hole available by appending groups that are binded M^{2+} ions (M^{2+} , ionic radii (Å): Ca^{2+} , 1.00; Cd^{2+} , 0.78; Mg^{2+} , 0.72; Co^{2+} , 0.65; Ni^{2+} , 0.44). (ii) The literature density functional theory (DFT) studies reported for the related Ca(II)-sensor complex [28] which highlighted that the Ca(II) ion is coordinated with eight oxygen atoms in the opened rings, while the other M(II) ions are coordinated in a six-chelation mode (*i.e.* closed), furthermore, electron-withdrawing groups (such as lutidinium group) are known to stabilize the ring-opened system and thus favoring Ca(II) ion binding.

3.6. Recycling of sensor

The new sensor exhibited a good reversibility as revealed by the restoration of its fluorescence performance upon the addition of ethylenediaminetetraacetate (EDTA), a very strong Ca(II) ion chelator. EDTA was interred in competition with our sensor for complexation with Ca(II) ions. By adding an equivalent amount of EDTA into the sensing system containing the probe and 10 mM of Ca(II) ions, fluorescence recovery as observed as a result of the BISBS-EDTA exchange to form Ca(II)-EDTA complex and the liberation of the BISBS probe. Thus, a reduction in fluorescence at 502 nm was observed until they reached 90% of their original intensity.

3.7. Quantum yield measurement

The quantum yield values were estimated for both free (BISBS) ligand and the metal complex to be 0.191 and 0.342 correspondingly. The estimated values were calculated by using quinine sulfate as probe of standard quantum yield [29], standard solution of 5×10^{-5} M quinine sulfate in sulfuric acid yields a quantum yield of 55%. The calculation of quantum yield is produced as following with limitation error for quantum yield ($\pm 7\%$) are predicted roughly.

$$Q_x = Q_R \frac{A_R \cdot I_S \cdot \eta_S^2}{A_S \cdot I_R \cdot \eta_R^2}$$

where

- R and S are the referencing solutions of BISBS or metal complex
- η is the solvents refractive index at 25 °C
- I is the area that integrated under fluorescence band
- A is the absorption maxima of all cases.

4. Conclusion

In conclusion, a new chemi-sensor (BISBS), *N,N'*-bis-[5-((2,4-lutidiniumchloride)-methylene)-3-methoxysalicylidene]-*R,R'*-1,2-cyclohexanedi-imine, based on fluorescence switch-on chemo-sensing for calcium metal ions was designed, synthesized and characterized. The novel bis-ionic Schiff base sensor displayed precious sensitivity and selectivity for sensing Ca(II) ions in comparison to different types of metal cations in aqueous solution. BISBS is applicable at physiological pH 7.6. The mechanism of detection process established with emission intensity enhancement which is depending on the internal charge transfer (ICT) mechanism in the way that Ca(II) cations acts as a receptor and BISBS Schiff base ligand acts as a donor. The new BISBS chemi-sensor consecutively provides low LOD 1.5 nM and significant detecting of Ca(II). Thus BISBS may provide a novel precious methodology for calcium cations detection in environmental and biological samples.

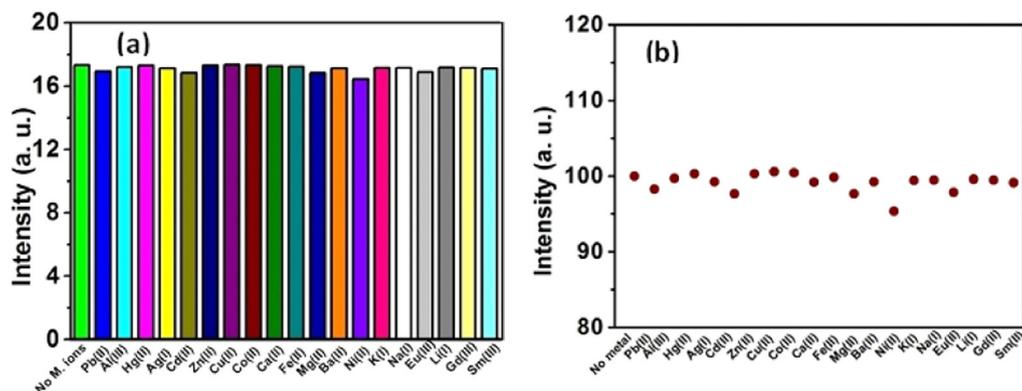


Fig. 7. (a, b) The fluorescence intensity of BISBS in presence of Ca(II) and different metal ions at 1.6 μM. Under excitation wavelength 380 nm and emission wavelength at 502 nm.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.molliq.2017.06.016>.

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