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(71) Applicant: **COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH** [IN/IN]; Anusandhan Bhawan, Rafi Marg, New Delhi 110001 (IN).

(72) Inventors: **DAS, Amitava**; National Chemical Laboratory, Dr. Homi Bhabha Road, Pune, Maharashtra 411008 (IN). **ALI, Firoj**; National Chemical Laboratory, Dr. Homi Bhabha Road, Pune, Maharashtra 411008 (IN). **SAHA, Sukdeb**; Analytical Science Discipline, CSIR-Central Salt & Marine Chemicals Research Institute, G.B. Marg, Bhavnagar, Gujarat 364002 (IN).

(74) Agents: **CHOPRA, Priyanka** et al.; K & S Partners, Intellectual Property Attorneys, 109, Sector 44, Gurgaon, National Capital Region 122003 (IN).

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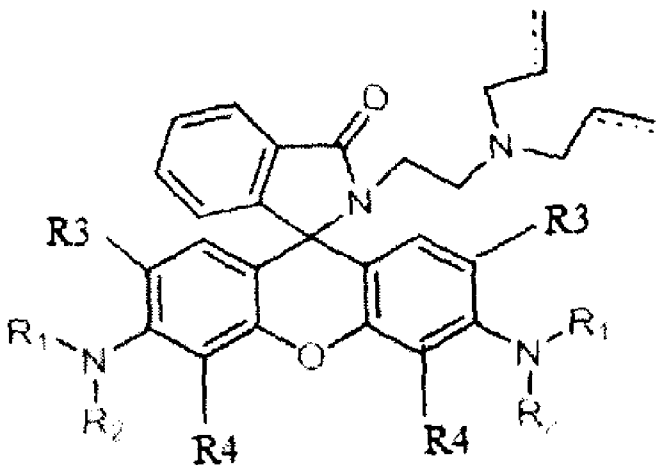
(54) Title: NOVEL LIGAND FOR DETECTION OF CHROMIUM (III) AND A PROCESS FOR THE PREPARATION THEREOF



Formula-I (Lx)

(57) Abstract: The invention disclosed herein relates to novel ligands (Lx) of Formula -I for selective detection of Cr (III) in pure aqueous medium and industrially viable process for the preparation thereof. Further the invention provides the process of selective detection of Cr (III) by fluorimetry using novel ligands of Formula-I. The invention also discloses a method of solubilizing novel ligands of formula-I in pure aqueous medium with the aid of non-ionic surfactant. The invention discloses a method of selective detection of Cr (III) using novel ligands of Formula-I.

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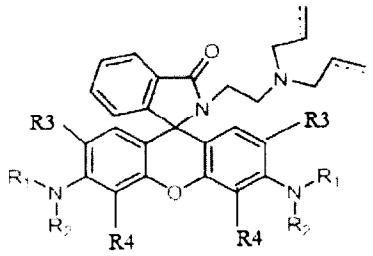
"NOVEL LIGAND FOR DETECTION OF CHROMIUM (III) AND A PROCESS FOR THE PREPARATION THEREOF"

FIELD OF THE INVENTION:

The invention discloses a novel ligand (L_x) of Formula-I with high selectivity to Cr (III) and a process for the preparation thereof. Particularly, the invention further discloses a method of determining Cr (III) in fluids, where the ligand should have solubility in aqueous medium with the aid of non-ionic surfactant.

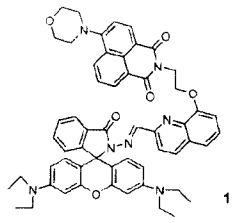


Formula-I (Lx)

BACKGROUND AND PRIOR ART OF THE INVENTION:

Due to its high paramagnetic behavior it is challenging for chemists to develop fluorogenic receptors specific for Cr (III) detection with *fluorescence on* response, as it is well known to quench the luminescence of a fluorophore. Further, due to the high solvation enthalpy of Cr (III)-ion in aqueous medium, it is also difficult to find an appropriate receptor for Cr(III -ion that works in pure aqueous medium.

There are only few examples of receptors available in the literature, which binds exclusively to Cr (III) in an ensemble of several other competing metal ions. The Receptor 1 was reported by Li *et.al*, Chem. commun, 2008, 3387. It is a FRET based chemo sensor for detection of Cr(III) in ethanol/water (2/1, v/v) medium and it can be used as an imaging agent in HeLa cell.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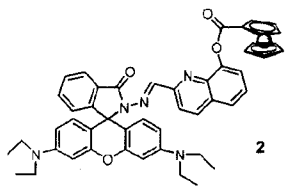
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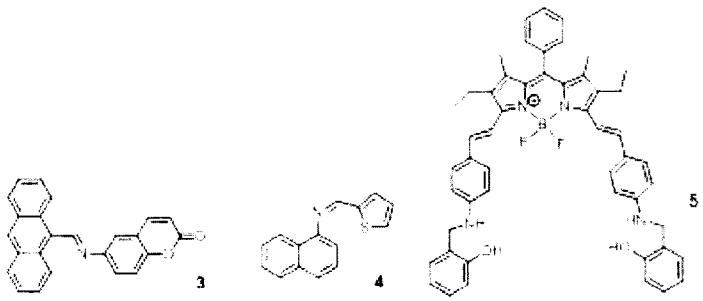
Li et al also reported another Ferrocene Based Receptor **2** (*Org. lett*, **2008**, 2557) for selective detection of Cr(III) in ethanol/water (1/1, v/v) with an association constant of 7.5 x $10^3 M^{-1}$. Receptors **3** (Anal. Methods, 2012, 3163; receptor is effective in acetonitrile/water (9/1, v/v)) and **4** (Anal. Methods, 2012, 2254; receptor is effective in methanol/water (9/1, v/v)) were developed by D. Das and his co-workers and both reagents showed high selectivity towards Cr(III) in predominantly organic medium. Receptor **5** is a BODIPY based Cr(III) sensors synthesised by D. Wang et al. (Tetrahedron Letters, **2010**, *51*, 2545). It can detect Cr(III) selectively in acetonitrile with binding stoichiometry of 2:2.



Further Hassan SS, et al. in *Analytical Sciences (Impact Factor: 1.57). 07/2005; 21(6):673-8* discloses use of a rhodamine-B chromate ion-associate complex as an electroactive material in a poly(vinyl chloride) membrane plasticized with o-nitrophenyloctyl ether as a solvent mediator. It is to be noted that the oxidation state for Chromium in chromate is (VI). They reported a Potentiometric Rhodamine B based Membrane Sensor for selective determination of Chromium ions [Cr(VI) & Cr(III)] in waste water. Firstly it can't detect Cr(III) directly. To detect Cr(III) they have adopted an indirect methodology in which Cr(III) was first oxidised to Cr(VI) by adding H_2O_2, that can be sensed by the reported sensor. Another important thing , this types of sensors cannot be applied for bio imaging application to monitor intra cellular Cr(III) activity.

In the present invention L_1 it can selectively detect only Cr(III) in pure aqueous medium. It does not have any interferences of Cr(VI) at all. It is a fluoromertic as well as colorimetric sensor for Cr(III) detection. It can be used for monitoring intra cellular imaging of Cr(III) . Fluorescence detection methods are more sensitive and simpler compare to other analytical methods.

Highly sensitive and selective fluorescence chemosensor for Cr^{3+} based on rhodamine B and a 4,13-diaza-18-crown 6-ether conjugate" is disclosed by *Duliang Liu, in RSC Adv., 26 Nov 2013,4, 2563-2567*, where detection of Cr^{3+} was possible only in predominantlty non aqueous environment (3:2, MeOH-H_2O (v/v).

There are only three previous reports that describe the use of reagents for the detection of Cr(III) in pure aqueous solution; (Mao, J et al. *Org. Lett.*, **2007**, *9*, 4567-4570 and Mao, J et al. *Anal. Bioanal. Chem.*, **2010**, *396*, 1197) one of them describes the interference by Fe^{3+}. The most recent report reveals that a rhodamine derivative within a polymeric matrix could be utilized for specific detection of Cr (III) in pure aqueous medium and the hydrophobic micro-environment generated around the binding core of the receptor induces a favourable influence for the detection of Cr(III) (*Macromol. Rapid Commun.* **2014**, 35, 323) However, the possibility of using these three molecular probes as an imaging reagent for studying the cellular uptake of Cr(III) is not explored and discussed.

While the receptors disclosed herein have the capability to detect Cr (III) in pure aqueous medium and in physiological condition. All other above examples suffer from a major drawback that they have this capability of detecting Cr (III) only in mixed aqueous organic solvents medium.

Detection of Cr (III) in pure aqueous solution is a very basic and vital need in the art, so that Cr(III) may be monitored for its presence and activity in cellular structure. There is also a need to detect Cr (III) in physiological fluids.

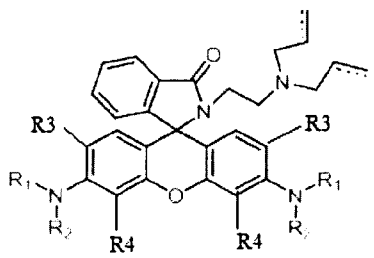
OBJECTS OF THE INVENTION:

Main objective of the present invention is to provide a novel ligand that could selectively detects Cr (III) in aqueous medium as well as in physiological pH (7.2).

Another objective of the invention is to develop a methodology for solubilizing the reagent in pure aqueous medium in presence of non-ionic surfactant like Titron X 100.

SUMMARY OF THE INVENTION:

Accordingly, the present invention provides novel ligands of Formula I (Lx) for detection of Chromium in pure aqueous medium



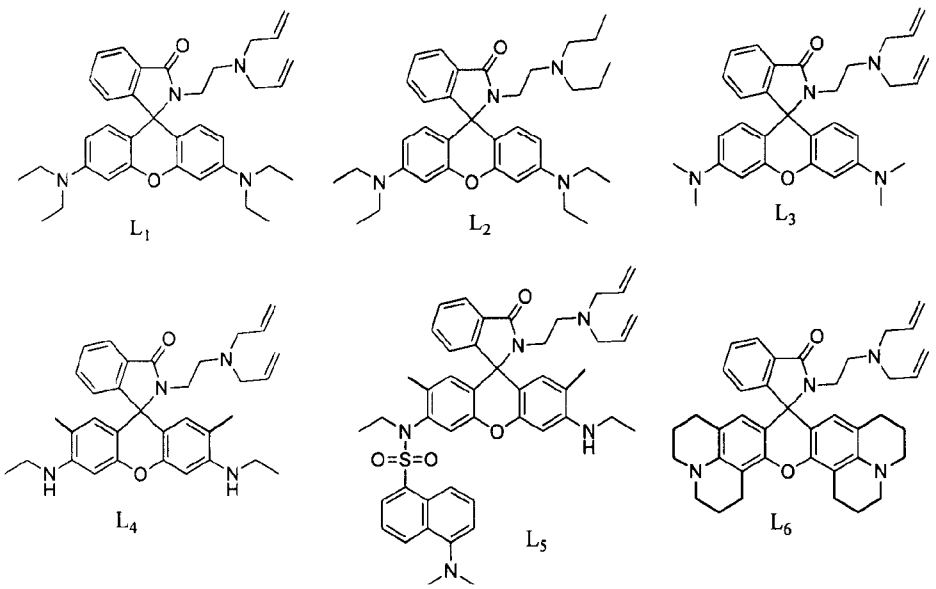
(Lx)

Formula I

wherein R_1 and R_2 are same or different and individually selected from the group consisting of H, linear or branched (C1-C6) alkyl, aryl or dansyl; R3 is same selected from group H, methyl; R4 is selected from H, (C1-C6) alkyl;

where, R1 may form saturated or unsaturated carbocyclic (C4-C6) ring with R3 and similarly R2 may form saturated or unsaturated carbocyclic (C4-C6) ring with R4; and(.....) line is optionally represents single bond.

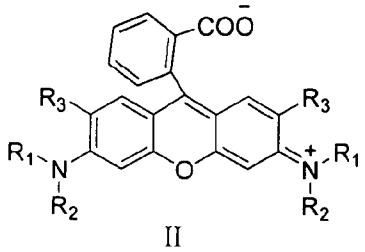
In one embodiment of the present invention the ligand of formula-I, encompasses the compounds selected from the group consisting of;



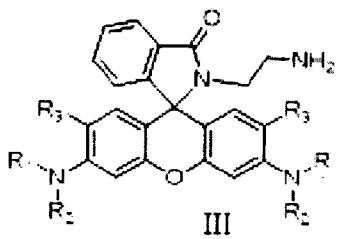
In an embodiment of the present invention a process of preparation of ligands of **Formula I**(Lx), wherein the said process comprising the steps of:

a. refluxing ethylene diamine and rhodamine B derivatives **(II)** in organic solvent, to obtain the corresponding amino ethylene rhodamine derivative **(III)** and;

b. refluxing the amino ethylene rhodamine derivative of step (a) in the presence of (A- Br) alliphatic bromide, triethyl amine and dry $CHCl_3$ under inert conditions to obtain Ligand (L_X) of Formula-I.



II



III

wherein R_1 and R_2 are same or different and individually selected from the group consisting of H, linear or branched (C1-C6) alkyl, aryl or dansyl; R3 is same selected from group H, methyl;

wherein, R1 may form saturated or unsaturated carbocyclic (C4-C6) ring with R3.

In another embodiment of the present invention the organic solvent is polar organic solvent selected from the group consisting of methanol, isopropanol, n-propanol, ethanol, water, butanol and mixtures thereof.

Still In another embodiment of the present invention the alliphatic bromide (A-Br) is alkene bromide selected from the group consisting of allyl bromide, 3-bromoprop-1-ene or alkyl bromide selected from the group consisting of propyl bromide, 1-bromopropane.

Still In another embodiment of the present invention a process for selective detection of Cr (III) using ligands of Formula-I, in aqueous medium as well as in physiological liquid of pH (7.2) comprising steps of:

a. preparing a solution of tris(hydroxymethyl)aminomethane buffer (Tris buffer)and Polyethylene glycol *tert*-octylphenyl ether (Triton X 100) at pH 7.2;

b. preparing a stock solution of ligands of Formula-I in a water miscible solvent in concentration ranges from 6.0 to 8.0 x 10^{-4} M;

c. mixing solution of step (b) with the solution of step (a) to solubilize ligand of formula I;

d. preparing Chromium (III) metal stock solution using water;

e. adding metal solution gradually to the solution of step (c) and;

f. recording spectrum in a UV or fluorescence spectrometer.

Still In another embodiment of the present invention the water miscible solvent is selected from acetonitrile, Methanol, DMSO, Ethanol, THF, DMF and mixtures thereof.

Still In another embodiment of the present invention a kit for selective detection of Cr (III) using novel ligands of Formula-I, comprising

a) Ligand L_1 stock solution (6.9×10^{-4} M) in acetonitrile;

b) 0.32 mM Triton X 100 in Tris buffer solution at pH 7.2;

c) Aqueous Cr(III) solution (3.28×10^{-3}M);

d) Final ligand solution (1.59×10^{-5} M) in 0.32 mM Triton X 100 in Tris buffer having solution pH of 7.2.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure 1 depicts ^{1}H NMR Spectra of ligand L_1

Figure 2 depicts ^{13}C NMR of L_1

Figure 3 depicts Mass Spectrum of L_1

Figure 4 depicts Emission titration of L_1 with aqueous Cr (III) solution in 0.4 mM Triton X in Tris PH 7.2. B-H plot. Binding constant (K) = $2.83 \times 10^3 M^{-1}$.

Figure 5: Benesi-Hildebrand (B-H) plots of emission spectral titration. All studies were performed in aq. solution of 0.4 mM Triton X-100 and Tris buffer (5 mM, 25 mM NaCl; pH 7.2).

Figure 6: depicts Bar diagram showing emission change at λ = 582 nm upon addition of various metal ions (1.0×10^{-4} M) to 15.6 μM of L_1 in 0.23mM Triton X 100 in Tris Buffer PH 7.2.

Figure 7 depicts UV Titration of Ligand L_1 (1.59×10^{-5} M) upon addition of aqueous Cr (III) solution in (0.4) mM Triton X 100 in Tris buffer having solution pH 7.2

Figure 8: depicts Flow Chart for detection procedure from a kit

Figure 9 :depicts Changes in (A) absorption and (B) emission spectra (λ_{Ext} of 530 nm) of the receptor L_1 (1.59×10^{-5} M) in absence and presence of different metal ions (M^{n+}: 1.62×10^{-4}M: Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Fe^{2+}, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+}); All studies were performed in aq. solution of 0.4 mM TX100 and Tris buffer (5 mM, 25 mM NaCl; pH 7.2).

Figure 10 depicts Isothermal Titration Calorimetry (ITC) titration profile for the binding of Cr^{3+} to receptor L_1 at 25°C in acetonitrile; Top plot: raw data for the sequential 2 μl injection of Cr^{3+} (1.2 x

10^{-3} M) into solution of L_1 (2.0×10^{-4} M) and bottom plot of the heat evolved (kcal per mole) of Cr^{3+} added.

Fig. 11: Plot of (I-I0) vs. [Cr3+], where I0 and I are emission intensities at 583nm of receptor L1 in the absence and presence of known [Cr3+] as well in tap water spiked with a known amount of Cr3+.

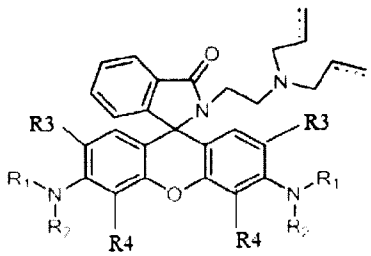
DETAILED DESCRIPTION OF THE INVENTION:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and appreciated.

For the purpose of this invention, the expression 'Novel Ligand (Lx)' or 'novel reagent' or 'receptor' 'colorimetric' as well as 'fluorescent chemosensor' are used interchangeably throughout the specification and the same may be appreciated as such by the person skilled in the art.

The present invention discloses a novel ligand for selective detection of Cr (III) in aqueous medium.

The novel ligand (L_x) of **Formula I** is as disclosed herein:



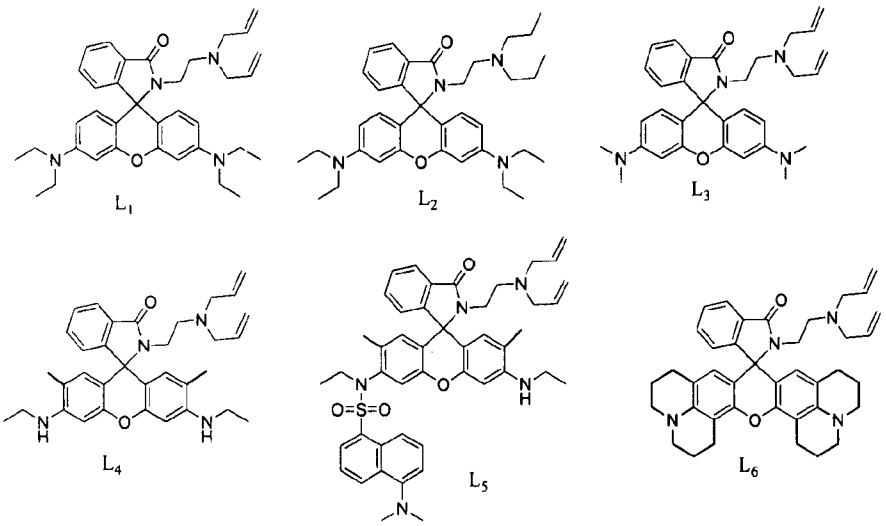
Formula I (Lx)

wherein R_1 and R_2 are same or different and individually selected from the group consisting of H, linear or branched (C1-C6) alkyl, aryl or dansyl; R3 is same selected from group H, methyl; R4 is selected from H, (C1-C6) alkyl;

where, R1 may form saturated or unsaturated carbocyclic (C4-C6) ring with R3 and similarly R2 may form saturated or unsaturated carbocyclic (C4-C6) ring with R4; and (.....) line is optionally represents single bond.

In another preferred embodiment, the invention provides the library of compounds of Formula-I.

The novel ligand (Lx) of Formula-I encompasses the compounds selected from the group consisting of;

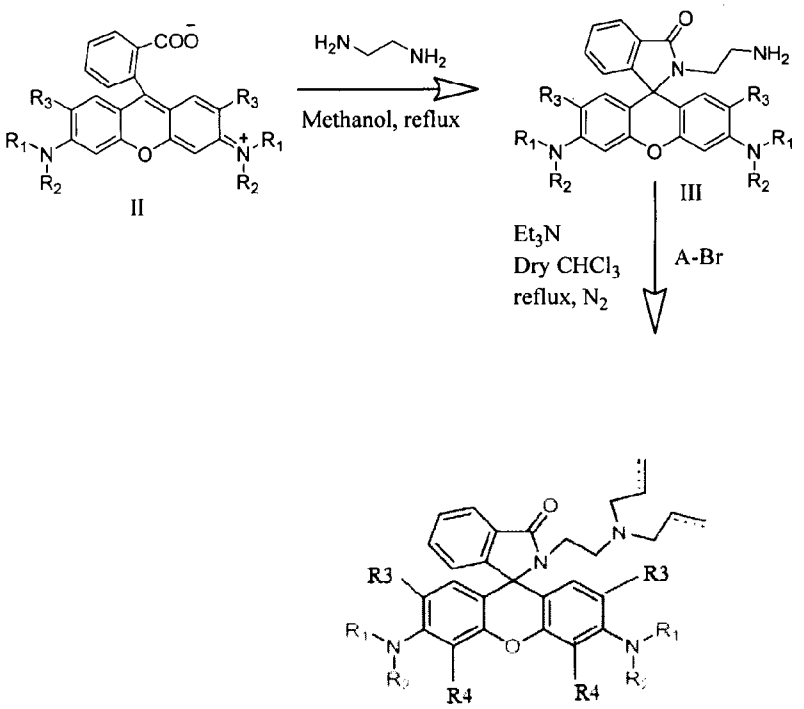


The invention provides a process of preparation of novel ligands of **Formula I** (Lx) comprising steps of:

a. refluxing ethylene diamine and rhodamine B derivatives (II) in an organic solvent, to obtain the corresponding amino ethylene rhodamine derivative **(III)** and;

b. refluxing the amino ethylene rhodamine derivative of step (a) in the presence of (A-Br) alliphatic bromide, triethyl amine and dry $CHCl_3$ under inert conditions to obtain Ligand L_X of Formula-I in good yield. (cf scheme 1)

Scheme 1;



According to the process, the organic solvent is polar organic solvent selected from the group consisting of methanol, isopropanol, n-propanol, ethanol, water, butanol and mixtures thereof.

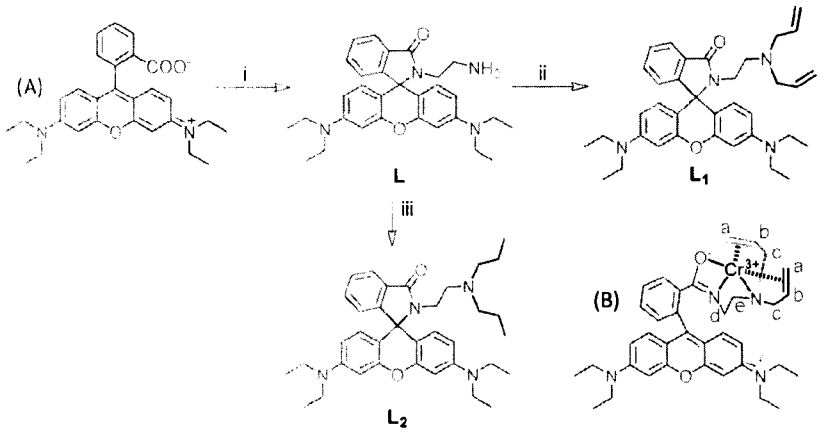
The alliphatic bromide (A-Br) is preferably alkene or alkyl bromide such as, allyl bromide, propyl bromide, 1-bromopropane, 3-bromoprop-1-ene.

The said process is time saving due to fewer steps and industrially feasible. The invention provides a process of preparation of novel ligands **(L_1)** and **(L_2)** comprising steps of:

a. refluxing ethylene diamine and rhodamine B in methanol to obtain the corresponding amino ethylene rhodamine derivative **(L)**; and
b. refluxing the amino ethylene rhodamine derivative of step (a) in the presence of allyl bromide, triethyl amine and dry $CHCl_3$ under inert conditions to obtain novel Ligand L_1; or

c. refluxing the amino ethylene rhodamine derivative of step (a) in the presence of propyl bromide, triethyl amine and dry $CHCl_3$ under inert conditions to obtain novel Ligand L_2. (refer scheme 2)

Scheme : 2 (A) Methodology adopted for synthesis of L_1 and L_2. (B) molecular structure for $Cr^{3+}.L_1$.



i. $H_2NCH_2CH_2NH_2$, MeOH, Reflux
ii. Dry $CHCl_3$, $H_2C{=}CHCH_2Br$, Et_3N, Inert Atmosphere.
iii. Dry $CHCl_3$, $H_2CCH_2CH_2Br$, Et_3N, Inert Atmosphere

According to the process, the Intermediate compound L was synthesized following a literature procedure, (A. *Org. Lett.* **2008**, *10*, 3013-3016). Methodologies that were adopted for synthesis of the receptor L_1 and the model compound L_2 are presented in the scheme 2. Desired compound L_1 and L_2 were isolated in pure form after necessary workup and were thoroughly characterized by various analytical/spectroscopic techniques.

The invention discloses a process for solubilisation of ligand L_1 in an aqueous medium employing non ionic surfactant, preferably Polyethylene glycol *tert*-octylphenyl ether (Triton X 100).

With reference to Figure 5 & Figure 7, Ligand L_1, dissolved in pure aqueous medium with the aid of Triton X 100, is selective/specific for Cr (III) and excludes similar metals selected from alkali, alkaline earth metals and all common transition metals.

In another preferred embodiment, the alkali, alkaline earth metals and transition metals are selected from, but not limited to Li, Na, K, Cs, Mg, Ca, Ba, Sr, Zn, Co, Cu, Ni, Fe, Pb, Hg and such like.

In another embodiment, the invention discloses the process of selective detection of Cr (III) by a process of fluorimetry comprising:

a. preparing a solution of Tris (tris(hydroxymethyl)aminomethane buffer)buffer and Triton X 100 at pH 7.2;

b. preparing a stock solution Ligand L_1 in a water miscible solvent; add required volume of Ligand stock solution to make desired Ligand L_1 concentration;

c. mixing Ligand L_1 solution of step (b) with the solution of step (a);

d. preparing metal stock solution using water;

e. adding metal solution gradually to the solution of step (c) and;

f. recording spectrum in a UV or fluorescence spectrometer.

According to the process the water miscible solvent is selected from acetonitrile, Methanol, DMSO, Ethanol, THF, DMF and mixtures thereof.

According to the process a stock solution of the receptor /ligand L_1 was prepared in acetonitrile medium with concentration ranges from 6.0 to 8.0 x 10^{-4} M, preferably (6.9 x 10^{-4} M);and the final concentration of metal salts is in the range of 1.0 to 2.0 x 10^{-4} M, preferably 1.62 x 10^{-4} M.

The UV-vis spectrum for the ligand L_1 may be carried out from for the range 250-800 nm and a λ_{max} of 562 nm is observed. The luminescence studies were carried out using an excitation wavelength of 530 nm and emission spectrum was monitored from 540 to 800 nm, with slit width of 2/2 nm; while a spectrum with λ_{max} of 583 nm is observed.

The invention provides a kit for selective detection of Cr (III) is disclosed. a kit for selective detection of Cr (III) comprises:

a) Ligand L_1 stock solution in acetonitrile.

b) 0.32 mM Triton X 100 in Tris buffer solution at pH 7.2.

c) Aqueous Cr (III) solution (3.28×10^{-3}M).

d) Final ligand solution (1.59×10^{-5} M) in (0.4) mM Triton X 100 in Tris buffer having solution pH of 7.2.

It describes the method of detection of Cr(III) in an aqueous medium

Further the invention provides a method of separation Cr (III) selectively from a sample comprising other metals, or other ingredients employing the ligand of Formula I described herein. It is possible to extract Cr (III) from aqueous solution when the concentration of Cr (III) either equal to or higher than 8.0×10^{-4}M. For extraction studies, dichloromethane (CH_2Cl_2) was used as the water immiscible organic solvent for extraction of Cr (III) from aqueous layer in the form of $L_1.Cr^{3+}$.

Experimental:

Materials and method:

Rhodamine B, Ethylenediamine, 3-bromoprop-1-ene, 1-bromopropane, all metal perchlorate salts (e.g $LiClO_4$, $NaClO_4$, $KClO_4$, $CsClO_4$, $Mg(ClO_4)_2$, $Ca(ClO_4)_2$, $Ba(ClO_4)_2$, $Sr(ClO_4)_2$, $Cu(ClO_4)_2$, $Zn(ClO_4)_2$, $Co(ClO_4)_2$, $Ni(ClO_4)_2$, $Cr(ClO_4)_3$, $Fe(ClO_4)_2$, $Cd(ClO_4)_2$, $Hg(ClO_4)_2$, and $Pb(ClO_4)_2$) and lanthanide ions as nitrate salts were obtained from Sigma-Aldrich and were used as received. Et_3N, Triton X-100, Tris Buffer, NaCl was procured from S.D. fine chemicals, India and was used as received. Solvents such as acetonitrile, chloroform were also purchased from S.D. Fine Chemicals, India and were used without further purification unless mentioned otherwise. Silica gel 100-200 mesh was used for column chromatography. Analytical thin layer chromatography was performed using silica Gel GF 254. HPLC grade water (Merck, India) was used for experiments and all spectral studies. Aminoethylene rhodamine B (L) was synthesized following a standard procedure (*Org. Lett.* **2008**, *10*, 3013-3016)

5.0 mM Tris-HCl aq. buffer solution was used for maintaining solution pH, unless mentioned otherwise. ESI-MS measurements were performed using a Micromass QTof- Micro instrument. FT-IR spectra were

recorded as KBr pellets using a Perkin Elmer Spectra GX 2000 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz FT NMR (model: Avance-DPX 500). Electronic spectra were recorded with a Varian Cary 500 Scan UV-Vis-NIR Spectrophotometer, Isothermal Titration Calorimetry studies were performed in Microcal iTC200, while emission spectra were recorded using either Edinburgh Instrument Xe-900 Spectrofluorometer or PTI. For all spectroscopic studies in aqueous buffer medium as well as for studies with plant/algal cells, L_1 self-assembled inside the miceller structure of TX100 was used, unless mentioned otherwise.

Photophysical study:

To check the selectivity of Receptor L_1 towards various metal ions like Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}, Co^{3+}, Cu^{2+}, Ni^{2+}, Fe^{2+}, Pb^{2+}, Hg^{2+} change in electronic as well as emission spectra were examined in 0.32 mM Triton X 100 in tris (5 mM, 25mM NaCl) buffer medium at PH 7.2.

Absorption and emission spectra of receptor L_1 in 0.32mM Triton X 100 in Tris buffer (5 mM , 25 mM NaCl) pH 7.2 medium shows no absorption band at 562 nm and very weak emission band at 583 nm on excitation at 562 nm. This solution of L_1 appears colourless and all these clearly suggest that L_1 in this solution is present exclusively in spirolactam form. This is also confirmed from the ^{13}C NMR studies, which shows a characteristic signal at 64.85 ppm for the tertiary C-atom. However only in presence of Cr^{3+}, a strong absorption band at 562 nm and intense emission band at 583 nm (for λ_{ext} = 530 nm) are observed. These changes are also associated with simultaneous visually detectable change in solution colour from colourless to pink red. For other metal ions, no such changes are observed.

For calculating the binding affinity of ligand L_1 (1.59×10^{-5} M) towards Cr^{3+} in aqueous solution, systematic absorption and emission spectral titration is performed in 0.32 mM Triton X 100 in tris buffer (5 mM, 25 mM NaCl) medium at PH 7.2. The association constant (K_{abs}) of 2.4×10^3 M^{-1} for the $L_1.Cr^{3+}$ formation is evaluated from the absorption titration and B-H plot data (Figure 3). A good linear fit data supported the 1:1 binding stoichiometry. For emission titration, a significant increase in emission intensity at 583 nm is observed with an increase in concentration of Cr^{3+} in aqueous medium. The binding affinity towards Cr^{3+} is also evaluated from linear fit B-H plot for 1:1 stoichiometry and is

found to be 2.83x 10^3 M^{-1}. The 1:1 binding stoichiometry is further confirmed from Jobs plot and presence of signal for m/z at at 616.87 (Calc 616.75) in FAB-MS analysis of a mixture of L_1 (1.59 x 10-5 M) and (9.5 x 10^{-5} M) $Cr(ClO_4)_3$ for [L_1 + Cr^{3+}].

To understand the role of Triton X 100 surfactant in the sensing of Cr^{3+}, the emission intensity of receptor L_1 (1.59 X 10^{-5} M) in presence of aqueous Cr^{3+} (5 mole eqv.) solution was plotted against different concentration of Triton X 100. The figure shows that sensing efficiency of the reagent described above is maximum when [Triton X] = 0.4 mM. This is also a good agreement with the data obtained from photo physical study shown in table below. Thus the surfactant concentration is adjusted to achieve maximum sensing efficiency of receptor L_1.

Table: Effect of Triton X 100 on binding affinity of L_1 to Cr^{3+} in aqueous medium.

[Titron X 100] (mM)	Binding Constant (M^{-1})		Quantum yield in water w.r.t. Rhodamine B
	Uv-Vis Spectroscopic Method	Fluorescence Spectroscopic Method	
0.23	1.96 x 10^3	2.0 x 10^3	0.185
0.32	2.4 x 10^3	2.83 x 10^3	0.211
0.4	3.2 x 10^3	3.25 x 10^3	0.22

UV-Vis and Fluorescence studies:

A solution of the perchlorate salts of the respective ion (Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Ba^{2+}, Zn^{2+}, Sr^{2+}, Hg^{2+} and Cr^{3+}) and nitrate salts of lanthanides ions (Tb^{3+}, Ho^{3+}, Ce^{3+}, Sm^{3+}, Rb^+, Pr^{3+}, Eu^{3+}, Gd^{3+}, Nd^{3+}, Dy^{3+}, Tm^{3+}, Er^{3+}, Yb^{3+}) in pure aqueous medium were used for all studies. The effective final concentrations of all metal salts were maintained at 1.62 x 10^{-4} M.

A stock solution of the receptor L_1 (6.9×10^{-4} M) was prepared in acetonitrile medium and 57 µL of this stock solution was added to 2.5 ml of 0.4 mM TX100 in Tris-HCl aqueous buffer medium having solution pH 7.2 to make the effective ligand concentration of 1.59×10^{-5} M. The solution was used for all the photophysical studies. Cr^{3+} stock solution (4.75×10^{-3} M) was prepared in pure aqueous medium and was used for all studies. Emission titrations were also performed as a function of [TX100] (0.1 mM, 0.23 mM, 0.32 mM, 0.4 mM, 0.6 mM) in Tris-HCl buffer medium of pH 7.2 by monitoring the increase in emission intensity (using λ_{Ext} = 530 nm, λ_{Mon} = 583 nm and slit width 2/2 nm) on binding of L_1 to Cr^{3+} for optimizing the maximum enhancement of the emission intensity. The relative fluorescence quantum yields (ϕ_f) were estimated using equation 1 for different concentration of TX100 (0.1 mM, 0.23 mM, 0.32 mM, 0.4 mM, 0.6 mM) in Tris-HCl buffer medium (having solution pH of 7.2) and by using the Rhodamine B (ϕ_f = 0.3 in aqueous medium at RT) as a reference.

$$\phi_f = \phi_f' \, (I_{sample}/I_{std})(A_{std}/A_{sample})(\eta^2_{sample}/\eta^2_{std}) \qquad \text{...Eq. 1}$$

where, ϕ_f' was the absolute quantum yield for the rhodamine B and was used as reference; I_{sample} and I_{std} are the integrated emission intensities; A_{sample} and A_{std} are the absorbances at the excitation wavelength, and η_{sample} and η_{std} are the respective refractive indices.

Computational Methodology:

The geometry of the compounds of Formula-I with chromium ion was examined by using known theories.The optimized geometry shows that the Cr^{3+} ion is coordinated with two nitrogen atoms, one oxygen atom, two olefinic π-bonds and a water molecule.

According to the invention, Figure 11 shows that among all the metal cations, only in the presence of Cr(III), respective absorption and emission (with λ_{Ext} = 530 nm) spectral bands at 562 nm and 583 nm were observed.

Formation of $Cr^{3+}.L_1$ was also confirmed from the result "Formation of Cr3+.L1 was also confirmed from the result of FAB MS study. Signal at m/z value of 616.87(Calc 616.75) for Cr3+.L1". Affinity of L_1 towards Cr(III) and the associated binding constant for the formation of $Cr^{3+}.L_1$ in aq. buffer medium

(pH = 7.2) was evaluated from the data obtained from B-H plots of the systematic absorption (K_a^{Abs} = 3.2 ± 0.2). 10^3 M^{-1}) as well as emission (K_a^{Ems} = (3.3 ± 0.2).10^3 M^{-1}) spectral titrations.

To envisage the adverse role of high solvation enthalpy of Cr(III) in aqueous medium, similar Uv-vis and emission titrations were carried out in acetonitrile medium using ([Cr(III)]= (0 -1.93 x 10^{-4})M; and $[L_1]$=(1.59 x 10^{-5} M) in CH_3CN) and the evaluated formation constant for $Cr^{3+}.L_1$ in acetonitrile was (K_a^{Abs} = (1.1 ± 0.03).10^6 M^{-1}, K_a^{Ems} = (1.0 ± 0.02).10^6 M^{-1} using λ_{Ext}: 530 nm and λ_{Mon}: 583 nm for emission titration). The binding affinity of L_1 towards Cr^{3+} was evaluated as K_a^{ITC} = (1.6 ± 0.02).10^6 M^{-1} in acetonitrile medium at 25°C using ITC experiments. Comparison of the binding constants evaluated in pure aq. buffer medium and in acetonitrile clearly revealed the energy barrier imposed due to the unfavourable solvation of Cr^{3+} in aqueous medium and thus adversely affecting the affinity of L_1 towards Cr^{3+}. Thermodynamic parameters were obtained from ITC studies (ΔG (-(8.48 ± 0.02) kcal mol^{-1}), ΔH ((-20.8 ± 0.4) kcal mol^{-1}) and ΔS (-41.4 ± 0.05) cal mol^{-1}) (all symbols are used according to standard terminology). The higher -ΔH value revealed that binding was exclusively driven by enthalpy change; while small but negative entropy of binding supported the formation of the adduct $Cr^{3+}.L_1$.

FTIR Analysis:

FTIR spectra recorded for L_1 and $Cr^{3+}.L_1$, also revealed a distinct shift from 1617 cm^{-1} to 1587 cm^{-1} (ΔV = 30 cm^{-1}) for C=C stretching frequency . Blue shift in absorption of vinyl group in the FTIR spectrum on coordination to metal ion is reported earlier. This also supports the involvement of olefins in coordination to Cr(III)-centre and possible mode of binding of L_1 to Cr(III) is shown in Scheme 1C. No such shifts were observed either in ^1H NMR or in FTIR spectra of L_1 in presence of certain other metal ions and these metal ions were chosen based on their ability to bind to the model reagent L_2 .

Examples

The following examples are given by way of illustration of the working of the invention in actual practice and therefore should not be construed to limit the scope of the present invention.

Example 1:

Procedure of synthesis of aminoethyl rhodamine B (L):

Amino ethyl rhodamine B is prepared according to literature (J.-H. Soh, K. M. K. Swamy, S. K. Kim, S. Kim, S.-H. Lee, J. Yoon, Tetrahedron Lett., 2007, 48, 5966).

Rhodamine B (1.0 g, 2.26 mmol) is dissolved in 30 mL of ethanol. It is then heated to 70°C with constant starring. Then ethylene diamine (3 mL) is added to the reaction medium. It is then allowed to reflux at 75^{0}C for 12 hour. The solvent is removed under vacuum and is dissolved in diluted HCl. Then to this resulting solution, NaOH solution was added in a drop-wise manner until precipitation is complete. The resulting solution mixture is then filtered, washed with water and is further dried to achieve the desired compound, amino ethyl rhodamine derivative as light red colour solid.

Synthesis of Ligand L_1:

Amino ethyl rhodamine B (400 mg, 0.826 mmol) was dissolved in 20 mL dry chloroform. To this Et_3N (3 mL) was added and the resulting solution is kept under N_2 atmosphere for 20 minutes. Then Allyl bromide (530 µL, 5.9 mmol) was added into starring solution. It was kept under reflux condition at temp 65^{0}C with constant stirring for 12 h until all the starting materials are consumed. Then 10 mL of water was added to it. The organic Layer is collected and dried over anhydrous Na_2SO_4 before concentration. It was finally purified by column chromatography using silica gel as stationary phase and 10% ethyl acetate in hexane as eluent or mobile phase to isolate L_1 in pure form with 50% yield. ^{1}H NMR (500 MHz, $CDCl_3$, $SiMe_4$, J (Hz), δ ppm) : 7.80 (d, J= 4.6), 7.35 - 7.30 (m), 6.99 (d, J= 4.4), 6.35 - 6.27 (m), 6.16 (d, J= 8.8), 5.56 (td, J= 16.5, 6.5), 4.96 - 4.88 (m), 3.24 (dd, J= 13.7, 6.8), 3.18 - 3.09 (m), 2.85 (d, J= 6.2), 2.21 - 2.13 (m), 1.07 (t, J= 6.8). ^{13}C NMR (500 MHz, $CDCl_3$, $SiMe_4$, δ ppm): 167.70, 153.38, 148.70, 135.13, 132.22, 131.54, 128.98, 127.97, 123.78, 122.63, 117.53, 108.03, 105.58, 97.73, 64.85, 56.60, 50.46, 44.37, 37.75, 29.69, 12.60. ESI-MS (+ *ve* mode, m/z): 565.93 (M + H^+), Calc. for $C_{36}H_{44}N_4O_2$ is 564.76.

Example 2: Synthesis of Ligand L_2:

Amino ethyl rhodamine B (200 mg, 0.41 mmol) was dissolved in 15 mL dry chloroform. To this Et_3N (500 µL) was added and the resulting solution was stirred for 20 minutes under N_2 atmosphere. Then 1-bromopropane (120 µL, 1.35 mmol) was added and the resulting reaction mixture was refluxed at temp for 24h until all the starting materials were consumed (Checked by TLC at different time interval). After this reaction mixture was allowed to attain the room temperature, 10 mL of water was added. The organic layer, after drying over anhydrous Na_2SO_4, was collected and followed by the removal of chloroform under vacuum to yield the crude product. Column chromatography was performed using silica gel as stationary phase and 10% ethyl acetate in hexane as mobile phase for isolating L_2 in pure form with 40% yield. ^1H NMR (500 MHz, $CDCl_3$, $SiMe_4$, J (Hz), δ ppm): δ 7.81 (dd, 1H, J = 5.9, 2.6 , H_{18}), 7.37 (dd, 2H, J = 5.6, 3.0, H_{16}, H_{17}), 7.05 - 7.00 (m, 1H, H_{15}), 6.35 (s, 1H, H_{12}), 6.33 (s, 1H, H_2), 6.31 (d, 2H, J = 2.5, H_5, H_9), 6.19 (dd, 2H, J = 8.9, 2.6, H_4, H_{10}), 3.26 (q, 8H, J = 7.0, H_{29}, H_{31}, H_{33}, H_{35}), 3.09 (d, 2H, J = 5.8, H_{21}), 2.15 (s, 6H, H_{22}, H_{23}, H_{26}), 1.18 (d, 4H, J = 6.7, H_{24}, H_{27}), 1.09 (t, 12H, J = 7.0, H_{30}, H_{32}, H_{36}, H_{34}), 0.68 (t, 6H, J =6.8, H_{25}, H_{28}). ^{13}C NMR (125 MHz, $CDCl_3$, $SiMe_4$, δ ppm): 167.75, 153.77, 148.72, 148.25, 132.25, 129.18, 127.78, 123.93, 122.59, 108.48, 105.63, 97.94, 64.95, 56.78, 50.96, 44.34, 37.79, 37.47, 20.27, 12.54, 11.82. IR (KBr): ν_{max}/cm^{-1} = 1680. ESI-MS (+ve mode, m/z): 569.29 ($M + H^+$), Calc. for $C_{36}H_{44}N_4O_2$ is 568.79. Elemental Analysis: Calculated (C 76.02, H 8.51, N 9.85); Experimentally obtained (C 76.20, H 8.50, N 9.88).

Example 3: Solubilization of Ligand L_1:

Method of preparation of Ligand (L_1) and solubilization of L_1 in water using Triton X 100:

Final Ligand (L_1) concentration = 1.59×10^{-5} M

Concentration of Cr^{3+} in aqueous solution = 10^{-4} M

1. Make 10 mL of 7.092×10^{-4} M stock solution of ligand L_1 in pure HPLC grade acetonitrile (Dissolve 4 mg of ligand L_1 in 10 mL HPLC grade acetonitrile).

2. Make a solution of 0.32 mM Triton X 100 in Tris-HCl Buffer (5 mM , 25 mM NaCl , pH 7.2) *i.e* medium. It is better to make 50 mL Tris-HCl Buffer solution (5 mM tris Buffer, 25 mM NaCl) and adjust the PH to 7.2. Then add 9.34 µL (10mg) of Triton X 100 to 50 mL of Tris-HCl Buffer (pH 7.2) solution and stir the solution gently for 10 minutes just to homogenies (avoid vigorous shaking).

3. Take exactly 56 µL of ligand stocks solution and add to 2.5 mL of solvent solution (*i.e.* 0.32 mM Triton X 100 in Tris-HCl buffer solution of pH 7.2) to make 1.59×10^{-5} M of ligand (L_1) solution for studies.

Molecular weight of the Ligand = 564

Molecular weight of the Triton X 100 = 625

Density of Triton X 100 = 1.07 g/L

Molecular weight of the Tris buffer = 121.14

Example 3:

Method of detection of Cr(III) from pure aqueous solution:

3.28×10^{-3} M Cr(III) solution in water is prepared and then it is gradually added to a solution of the ligand L_1, where effective ligand concentration is 1.59×10^{-5} M in a Tris buffer with solution pH of 7.2 having 0.32 mM Triton X 100. Uv-vis and luminescence spectra are recorded in absence and presence of [Cr(III)]. In emission spectra a 200 fold increase in spectral intensity at 583 nm (λ_{ext} = 530 nm and slit 2/2) is observed, while a simultaneous change in solution colour is observed from colourless to pink red (please refer to Figures 4 and 6).

<u>Real sample analysis:</u>

To cheeked the applicability of the methods in real sample analysis , probe L1 was applied to detect Cr3+ in tap water. No Cr3+ was obtained in tap water samples. Water samples were collected and pH was adjusted to 7.2 using Tris buffer(10mM, 25mM NaCl), spiked with known (10µM and 20µM) concentration of Cr3+ and emission spectra was recorded. The result was summarised in Table 1.

Table 1: Determination of Cr^{3+} in tap water.

Sample No.	Cr^{3+} added (μM)	Cr^{3+} found (μM)	Recovery (%)
1	10	9.81	98.1
2	20	19.67	98.3

Advantages of the invention:

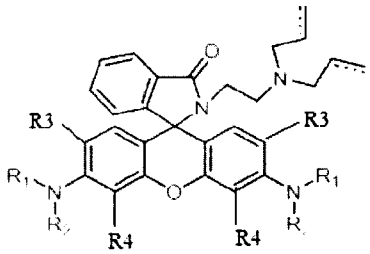
1. With this ligand (L_1), Cr(III) can be detected from pure aqueous solutions at physiological pH.

2. The ligand can also be used as a colorimetric as well as fluorescent chemosensor for the detection of Cr(III) in aqueous solutions.

3. The use of Triton X 100 to create micro-micellar environment that makes the ligand soluble in water or tri buffer medium having pH 7.2 cell membrane permeable in addition to maximizing the sensing efficiency.

The claim:

1. Novel ligands of Formula I (Lx) for detection of Chromium in pure aqueous medium.



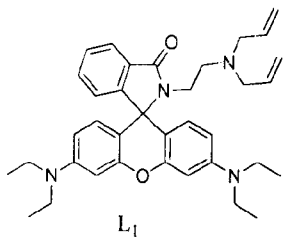
(Lx)

Formula I

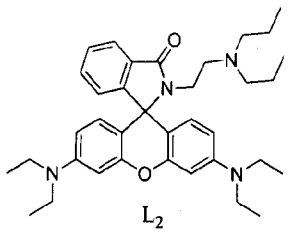
wherein R_1 and R_2 are same or different and individually selected from the group consisting of H, linear or branched (C1-C6) alkyl, aryl or dansyl; R3 is same selected from group H, methyl; R4 is selected from H, (C1-C6) alkyl;

where, R1 may form saturated or unsaturated carbocyclic (C4-C6) ring with R3 and similarly R2 may form saturated or unsaturated carbocyclic (C4-C6) ring with R4; and(.....) line is optionally represents single bond.

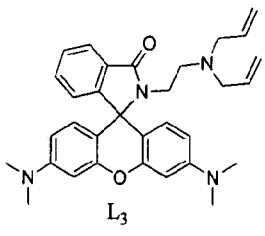
2. The ligand of formula-I, encompasses the compounds selected from the group consisting of;



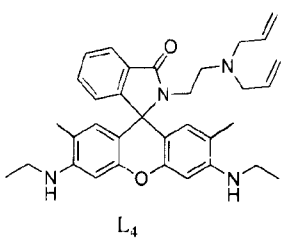
L_1



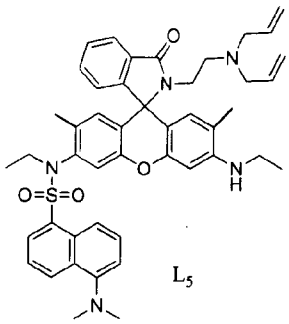
L_2



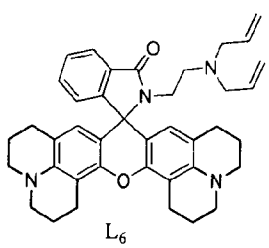
L_3



L_4



L_5

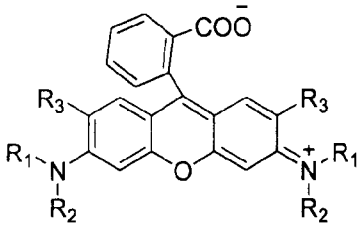


L_6

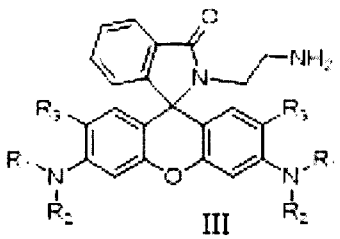
3. A process of preparation of ligands of **Formula I**(Lx) according to claim 1, wherein the said process comprising the steps of:

a. refluxing ethylene diamine and rhodamine B derivatives (**II**) in organic solvent, to obtain the corresponding amino ethylene rhodamine derivative (**III**) and;

b. refluxing the amino ethylene rhodamine derivative of step (a) in the presence of (A- Br) alliphatic bromide, triethyl amine and dry $CHCl_3$ under inert conditions to obtain Ligand (L_X) of Formula-I.



II



III

wherein R_1 and R_2 are same or different and individually selected from the group consisting of H, linear or branched (C1-C6) alkyl, aryl or dansyl; R3 is same selected from group H, methyl;
wherein, R1 may form saturated or unsaturated carbocyclic (C4-C6) ring with R3.

4. The process according to claim, 3, wherein the organic solvent is polar organic solvent selected from the group consisting of methanol, isopropanol, n-propanol, ethanol, water, butanol and mixtures thereof.

5. The process according to claim 3, wherein the alliphatic bromide (A-Br) is alkene bromide selected from the group consisting of allyl bromide, 3-bromoprop-1-ene or alkyl bromide selected from the group consisting of propyl bromide, 1-bromopropane.

6. A process for selective detection of Cr (III) using ligands of Formula-I according to claim 1, in aqueous medium as well as in physiological liquid of pH (7.2) comprising steps of:

a. preparing a solution of tris(hydroxymethyl)aminomethane buffer (Tris buffer)and Polyethylene glycol *tert*-octylphenyl ether (Triton X 100) at pH 7.2;

b. preparing a stock solution of ligands of Formula-I in a water miscible solvent in concentration ranges from 6.0 to 8.0 x 10^{-4} M;

c. mixing solution of step (b) with the solution of step (a) to solubilize ligand of formula I;

d. preparing Chromium (III) metal stock solution using water;

e. adding metal solution gradually to the solution of step (c) and;

f. recording spectrum in a UV or fluorescence spectrometer.

7. The process for selective detection of Cr (III) according to claim 7, wherein the water miscible solvent is selected from acetonitrile, Methanol, DMSO, Ethanol, THF, DMF and mixtures thereof.

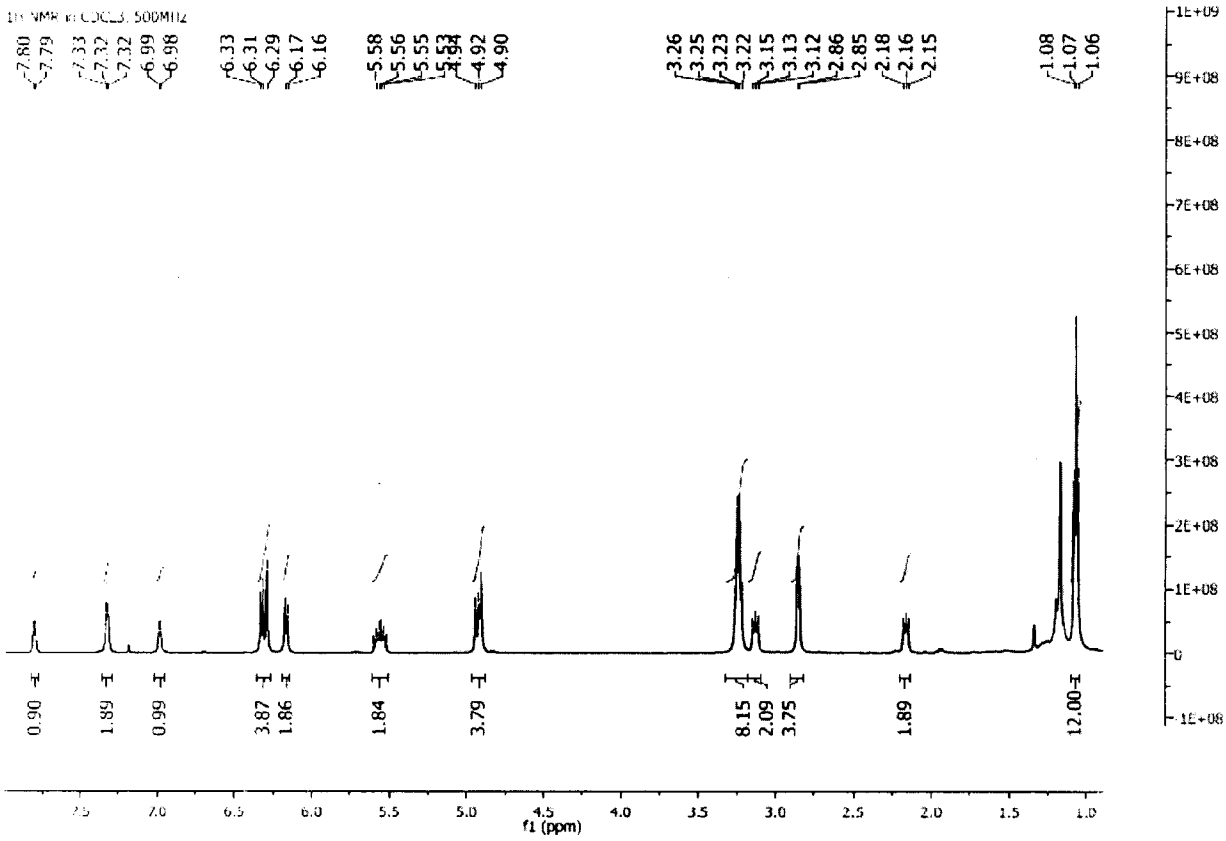
8. A kit for selective detection of Cr (III) using novel ligands of Formula-I according to claim 1, comprising

a) Ligand L_1 stock solution (6.9 x 10^{-4} M) in acetonitrile;

b) 0.32 mM Triton X 100 in Tris buffer solution at pH 7.2;

c) Aqueous Cr(III) solution (3.28 x 10^{-3}M);

d) Final ligand solution (1.59 x 10^{-5} M) in 0.32 mM Triton X 100 in Tris buffer having solution pH of 7.2.


7.80
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7.33
7.32
7.32
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6.98
6.33
6.31
6.29
6.17
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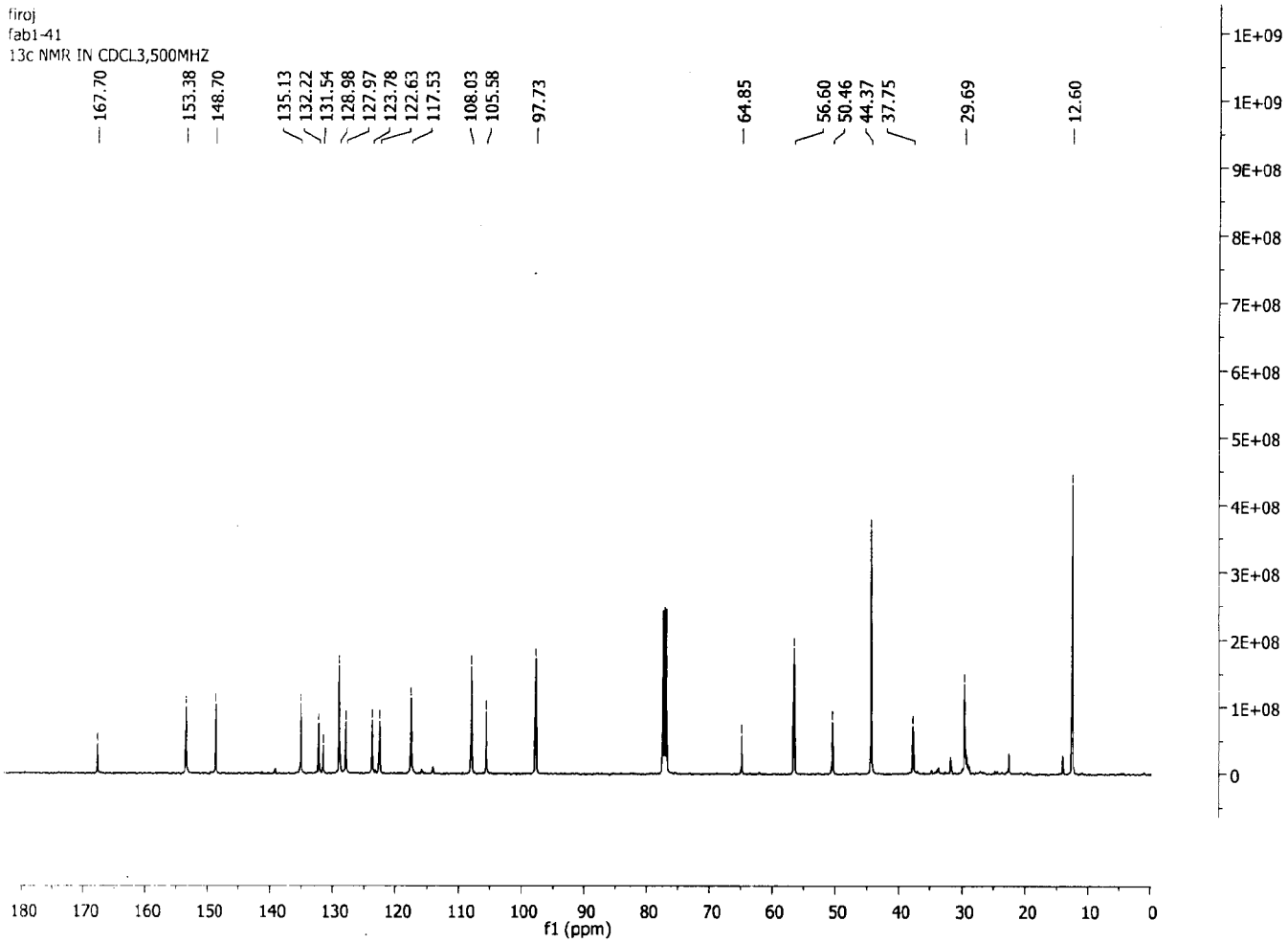
5.55
4.92
4.90
3.26
3.25
3.23
3.22
3.15
3.13
3.12
2.86
2.85
2.18
2.16
2.15
1.08
1.07
1.06
0.90
1.89
0.99
3.87
1.86
1.84
3.79
8.15
2.09
3.75
1.89
12.00
f1 (ppm)

Figure 1:


firoj
fab1-41
13c NMR IN CDCL3,500MHZ
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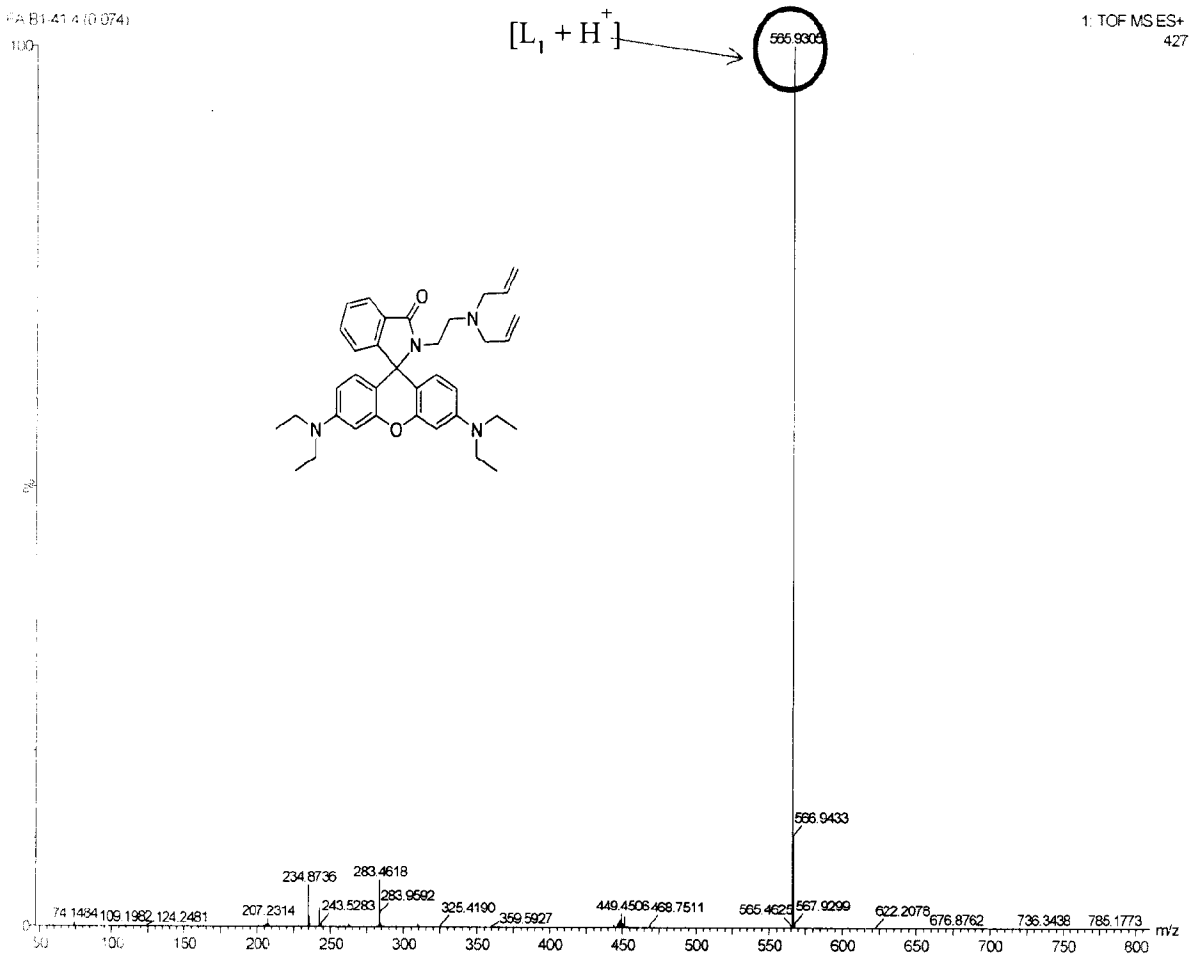
153.38
148.70
135.13
132.22
131.54
128.98
127.97
123.78
122.63
117.53
108.03
105.58
97.73
64.85
56.60
50.46
44.37
37.75
29.69
12.60
1E+09
1E+09
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


[L1 + H+]
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566.9433
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283.9592
74.1484
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124.2481
207.2314
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325.4190
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m/z

Figure 3:

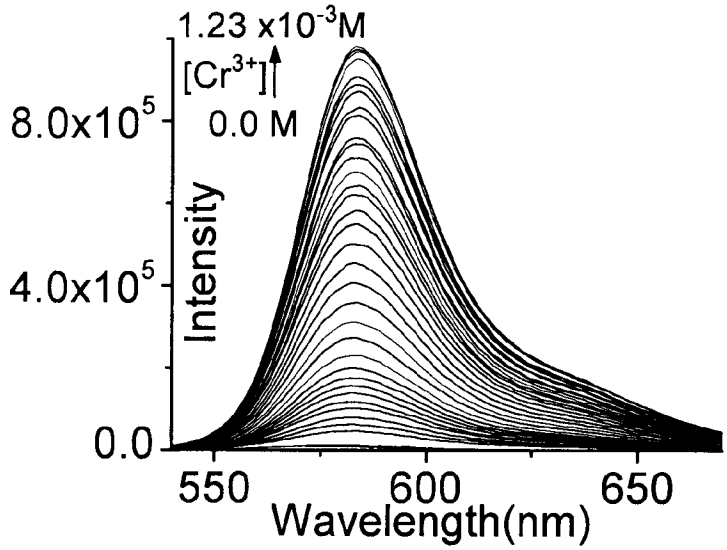


Figure 4:

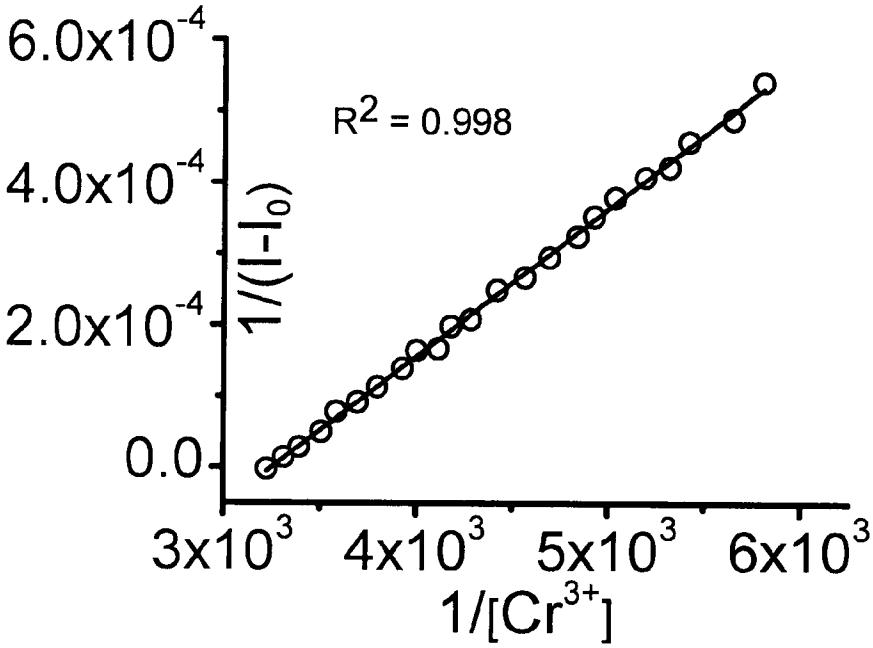


Figure 5:

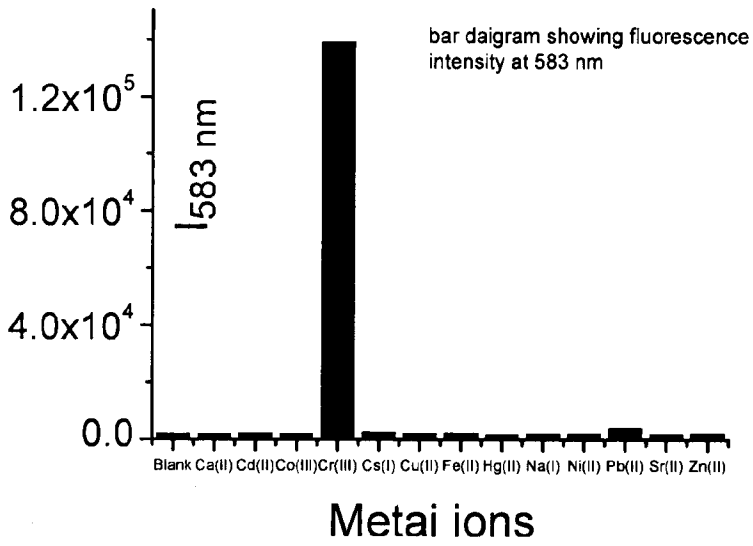


Figure 6:

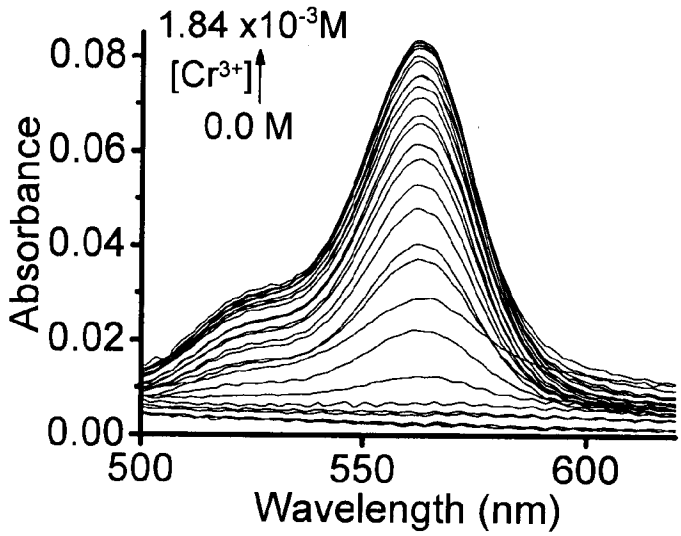
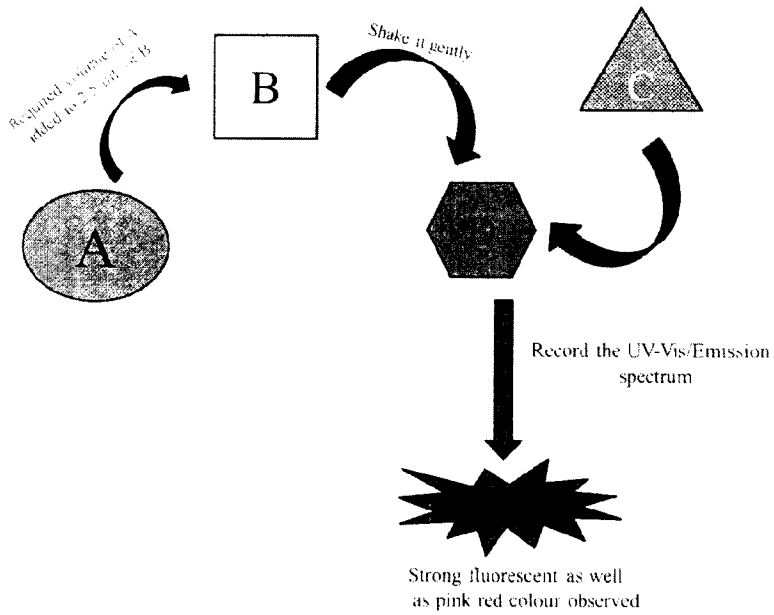


Figure 7:



= Final spectral changes as well as visual color change observed.

Figure 8:

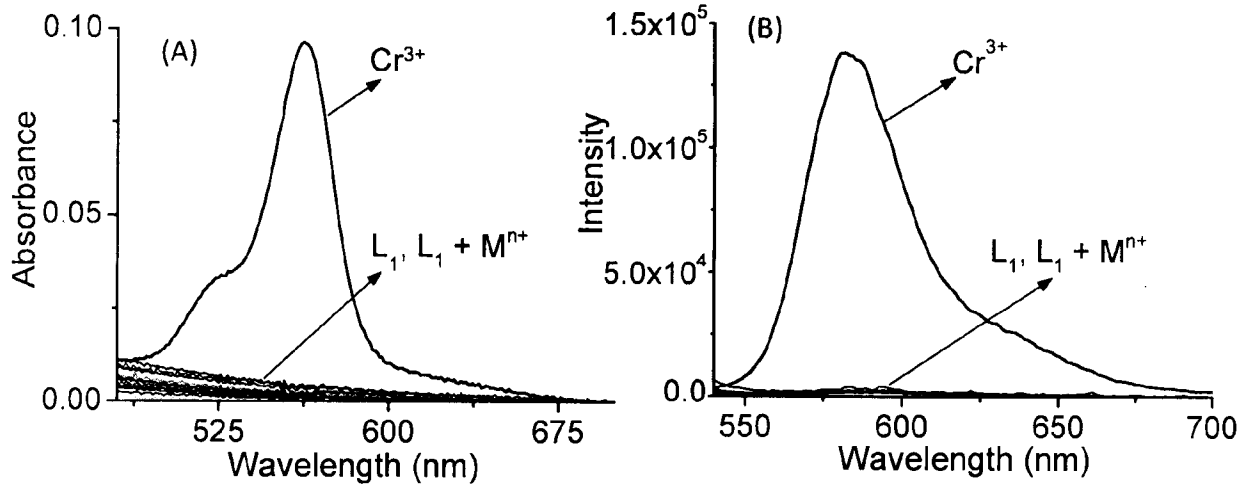


Figure 9:

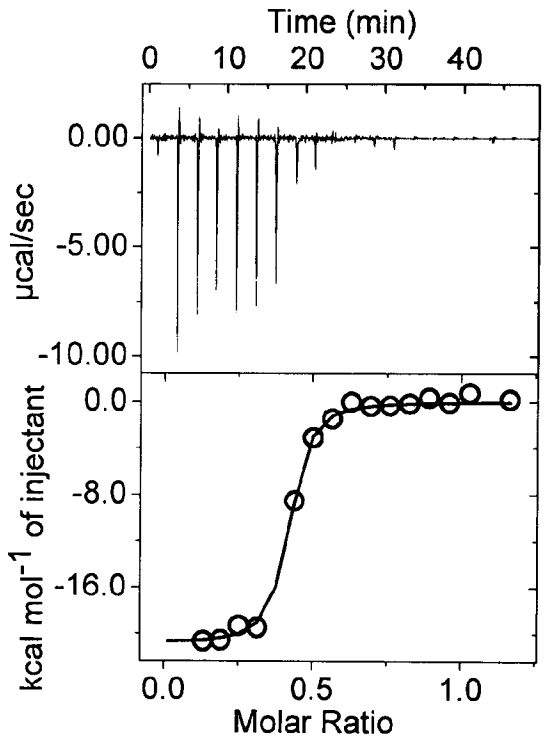


Figure 10:

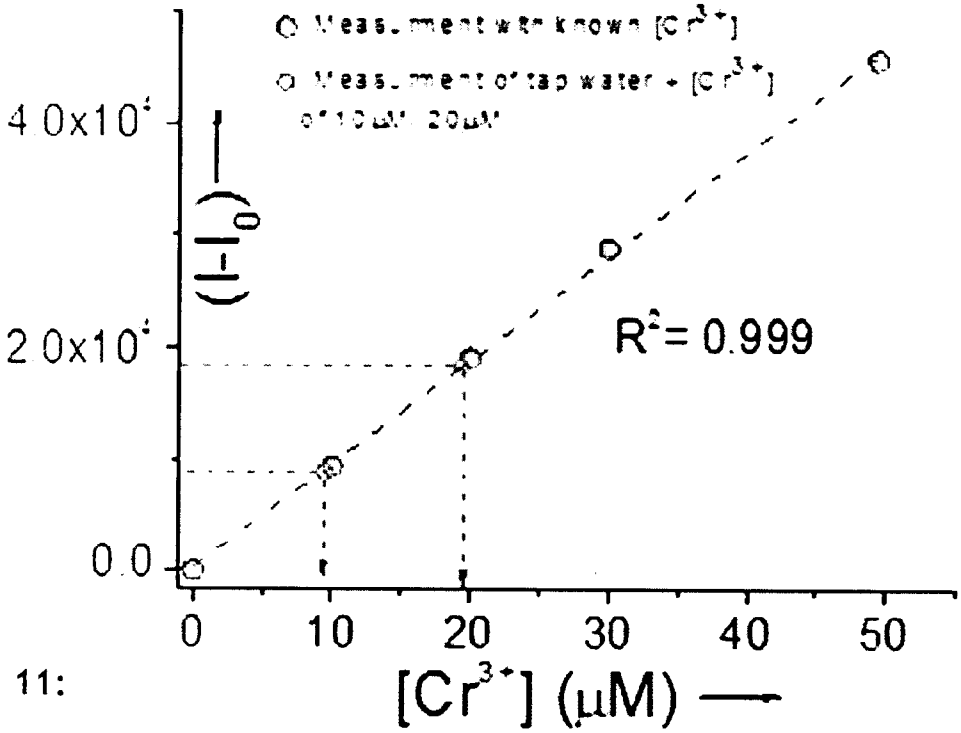

Measurement with known [Cr3+]
Measurement of tap water + [Cr3+]
4.0x10⁴
2.0x10⁴
0.0
R²= 0.999
0
10
20
30
40
50
[Cr3+] (μM)

Fig. 11:

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2014/000646

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D491/107 G01N21/64 G01N33/52
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. H. LEE ET. AL.: "Metal Ion Induced FRET OFF-ON in Tren/Dansyl-Appended Rhodamine", ORGANIC LETTERS, vol. 10, no. 2, 14 December 2007 (2007-12-14), pages 213-216, XP002734475, DOI: 10.1021/ol702558p page 214, Scheme 1, compound 4 ----- -/--	1-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2015	20/02/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Helps, Ian

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2014/000646

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Y. LEI ET. AL.: "Photophysical property of rhodamine-cored poly(amidoamine) dendrimers. Simultaneous effect of spirolactam ring-opening and PET process on sensing trivalent chromium ion.", JOURNAL OF LUMINESCENCE, vol. 131, 16 July 2011 (2011-07-16), pages 2521-2527, XP002734476, page 2525, column 1, paragraph 2 - page 2526, column 2, paragraph 1; figures 1-6	1-8
A	Z. ZHOU ET. AL.: "FRET Based Sensor for Imaging Chromium(III) in Living Cells.", CHEMICAL COMMUNICATIONS, vol. 2008, 23 May 2008 (2008-05-23), pages 3387-3389, XP002734477, DOI: 10.1039/b801503a cited in the application figures 1-5	1-8
A	J. MAO ET. AL.: "Tuning the selectivity of Two Chemosensors to Fe(III) and Cr(III).", ORGANIC LETTERS, vol. 9, no. 22, 27 September 2007 (2007-09-27), pages 4567-4570, XP002734478, DOI: 10.1021/ol7020687 cited in the application page 4568, Scheme 1, Figures 1-4.	1-8
A	J. MAO ET. AL.: "An "On-Off" Fluorescence Probe for Chromium (III) Ion Determination in Aqueous Solution.", ANALYTICAL AND BIOANALYTICAL CHEMISTRY, vol. 396, 22 December 2009 (2009-12-22), pages 1197-1203, XP002734479, cited in the application page 1199, paragraph 4 - page 1202, paragraph 2; figures 1-7	1-8

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