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(71) Applicants: INDIAN INSTITUTE OF SCIENCE

[IN/IN]; Bangalore - 560012, Karnataka, India., Bangalore 560012 (IN). ICAR - NATIONAL BUREAU OF AGRICULTURAL INSECT RESOURCES (NBAIR) [IN/IN]; P. B. No 2491, H.A. Farm Post, Bellary Road, Bangalore - 560 024, Karnataka 560 024 (IN).

(72) Inventors: BHATTACHARYA, Santanu; Department of

Organic Chemistry, Indian Institute of science, Bangalore, Karnataka 560012 (IN). BISWAKARMA, Dipen; Department of Organic Chemistry, Indian Institute of science, Bangalore, Karnataka 560012 (IN). DEY, Nilanjan; Department of Organic Chemistry, Indian Institute of science, Bangalore, Karnataka 560012 (IN). BHAGAT, Deepa; ICAR - National Bureau of Agricultural Insect Resources (NBAIR), P. B. No. 2491, Bangalore, Karnataka 560024 (IN).

(74) Agent: BHATTA, H L Narendra; C/O INTELLOCOPIA

IP SERVICES, 29, 'SHESHASRI', I 'C' CROSS, SN LAY-OUT, BASAVESWARNAGAR, BANGALORE-560079, Bangalore 560079 (IN).

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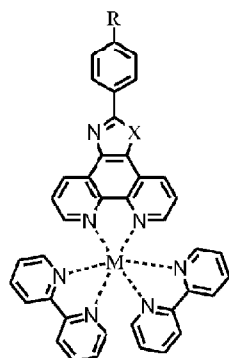
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FORMULA I

FIG. 1

(57) Abstract: The invention provides a compound for specific detection of SLNPV. Further, the invention provides a probe for specific detection of SLNPV. The probe comprises of a substrate embedded with the compound. The invention also provides a kit comprising a probe and detection means for specific detection of SLNPV.

A KIT FOR SPECIFIC DETECTION OF SLNPV

FIELD OF INVENTION

The invention generally relates to the field of biopesticide detection and
5 more particularly to a kit for specific detection of SLNPV.

BACKGROUND

Spodoptera litura, hereinafter referred to as *S. litura*, is one of the most harmful polyphagous pests known in India. It can feed on 112 species of
10 plants belonging to 44 different families worldwide and also on 60 different species of cultivated crops, reference of which is incorporated herein. In the Indian subcontinent, the insect shows high reproductive rate (i.e., 8-11 generations per year) with excellent adaptability towards the adverse weather conditions, reference of which is incorporated herein. Moreover,
15 *S. litura* is also reported to develop its resistance to several commercial insecticides, such as Malathion, Pyrethrum, Lindane and Endosulfan etc, reference of which is incorporated herein.

Various methods are known in the art that are adopted to control the *S. litura* caused infestation. Examples of commonly adopted methods include
20 use of sex pheromone traps, horticultural control, genetically improved plant varieties, biological control, chemical control and/or combinations thereof. One example of a biological control method employs the use of *Spodoptera Litura Nucleopolyhedroviruses* biopesticide, hereinafter referred to as SLNPV. The method includes spraying a water-dispersible
25 liquid Formulation of SLNPV to the infested produce. A significant advantage of the Formulation is that the Formulation is less hazardous to the environment. However, one significant disadvantage of the spray method is that the efficacy of the Formulation significantly reduces with time, due to decrease in the number of viable virus, present in the sprayed
30 Formulation. One of the techniques for determining efficacy of the SLNPV Formulation is the quantification of the occlusion bodies present in SLNPV Formulation.

One known method uses a Hemocytometer with improved Neubauer, for counting of virus particles. One essential criterion for accurate count of the virus particles, present in the sample, is that the sample should be devoid of contaminations. A further requirement includes a sample devoid of any aggregation and/or clumps of the virus particles. Additionally, the use of Hemocytometer also requires elaborate initial arrangement and calibration. Further, the material cost for conducting the experiment is high and the experiment requires strict maintenance of several parameters. Also, there is a requirement of skilled personnel to handle the Hemocytometer and to make accurate observations. Hence, there is a need for quantification of SLNPV in commercial Formulations which is rapid, cost-effective, easy and does not require any expert knowledge in handling.

BRIEF DESCRIPTION OF DRAWINGS

So that the manner in which the recited features of the invention can be understood in detail, some of the embodiments are illustrated in the appended drawings. It is to be noted, however, that the appended drawings illustrate only typical embodiments of this invention and are therefore not to be considered limiting of its scope, for the invention may admit to other equally effective embodiments.

FIG. 1 shows a representative structure of the compound I, according to an embodiment of the invention.

FIG. 2a shows a plot of emission spectra of compound I in the presence of SLNPV, as observed in water, according to an example of the invention.

FIG. 2b shows linear change in emission intensity of compound I at 605nm in the presence of SLNPV in water, according to an example of the invention.

FIG. 3a shows a plot of emission spectra of the compound I with SLNPV, in different pH ranges, according to an embodiment of the invention.

FIG. 3b shows a plot of emission spectra of the compound I with SLNPV, in different temperature ranges, according to an embodiment of the invention.

FIG. 4 shows effect of different adjuvants on emission intensity of compound I, according to an embodiment of the invention.

FIG. 5a shows a plot of emission spectra of heat treated SLNPV, according to an embodiment of the invention.

FIG. 5b shows a plot of CD spectra of heat treated SLNPV, according to an embodiment of the invention.

FIG. 6a shows plot of emission spectra of compound I with heat treated SLNPV, according to an embodiment of the invention.

FIG. 6b shows the comparison of compound I interaction with normal and heat treated SLNPV, according to an embodiment of the invention.

FIG. 7a shows a plot of fluorescence titration of compound I with HaNPV at pH 7.0 in water, according to an embodiment of the invention.

FIG. 7b shows a plot of change in emission intensity of compound I in presence of both HaNPV and SLNPV at pH 7.0 in water, according to an example of the invention.

FIG. 8 shows changes in thermodynamic parameters of compound I upon interaction with SLNPV, according to an embodiment of the invention.

FIG. 9a shows changes in zeta potential values of SLNPV upon interaction with compound I, according to an embodiment of the invention.

FIG. 9b shows changes in zeta potential values of SLNPV, upon interaction with the increasing concentration of compound I, according to an embodiment of the invention.

FIG. 10a shows the interaction of SLNPV with compound II, according to an embodiment of the invention.

FIG. 10b shows the change in emission intensities of compound I and compound II, in presence of SLNPV at pH 7.0, according to an embodiment of the invention.

FIG. 11 shows a graph depicting change in hydrodynamic diameter of compound I in presence of SLNPV at pH 7.0 in water, according to an example of the invention.

FIG. 12a shows circular dichroism spectra of SLNPV with compound I, according to an embodiment of the invention.

FIG. 12b shows FT-IR spectra of SLNPV with compound I, according to an
5 embodiment of the invention.

FIG. 13a shows a plot of emission spectra of the probe with a commercial Formulation of SLNPV, according to an embodiment of the invention.

FIG. 13b shows a recovery plot of the probe in presence of commercial Formulation of SLNPV at pH 7.0 in water.

10 FIG. 14 shows change in the red colour intensity and a graph showing the change in intensity against different concentrations of SLNPV, according to an embodiment of the invention.

FIG. 15 shows the change in the intensity of the red color plotted upon addition of different adjuvants of commercial Formulations of SLNPV,
15 according to an embodiment of the invention.

FIG. 16a shows change in the red colour intensity of the probe with fresh and heat treated SLNPV, according to an embodiment of the invention.

FIG. 16b shows change in the red colour intensity of the probe with pure and commercial mixture of SLNPV, according to an embodiment of the
20 invention.

FIG. 17 shows change in the red colour intensity of the probe upon addition of HaNPV and SLNPV and a plot showing the change in intensity of red colour when plotted against HaNPV and SLNPV, according to an embodiment of the invention.

25 FIG. 18a shows emission intensity of compound I with SLNPV in fruits, according to an example of the invention.

FIG. 18b shows emission intensity of compound I with SLNPV in vegetable extracts, according to an example of the invention.

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SUMMARY OF THE INVENTION

One aspect of the invention provides a compound for specific detection of SLNPV.

- 5 Another aspect of the invention provides a probe for specific detection of SLNPV.

Yet another aspect of the invention provides a kit comprising a probe and detection means for specific detection of SLNPV.

DETAIL DESCRIPTION OF THE INVENTION

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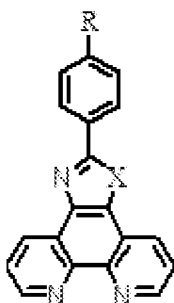
Various embodiments of the invention provide a method of measuring the insecticidal efficacy of SLNPV by quantifying the number of occlusion bodies present in its commercial Formulation. One embodiment of the invention provides a compound for specific detection of SLNPV. The compound for specific detection of SLNPV is represented by a structure of general Formula I, hereinafter referred to as compound I. FIG.1 shows a representative structure of compound I, according to an embodiment of the invention. The compound includes a metal ion M. In one example of the invention, the metal ion is a trivalent metal ion, examples of metal ion include but are not limited to Fe, Ru and Os. The element X, of the compound of Formula I is selected from a list including O, S and NH. The R group on the representative structure of the compound of Formula I is an O-alkyl. The compound as represented herein by the Formula I is obtained by a method as described herein below.

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25 SYNTHESIS OF COMPOUND I.

In order to obtain the compound I, initially a precursor molecule of Formula II is synthesized.

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FORMULA II

Synthesis of II: To a warm solution of R1 (0.23g, 1.01mmol) and acetate salt (0.722g, 9.36 mmol) in glacial acetic acid, RCHO (0.15g, 1.01mmol) is added. The resulting mixture is heated to 85°C for 5h and then cooled to room temperature. The cooled mixture was poured into 100mL of cold water and neutralized to pH 7 with an aqueous solution of ammonia. The precipitation so obtained was filtered off. The synthesized compound of Formula II is then utilized in the synthesis of compound of Formula I. A 1:1 solution of methanol-water is prepared. To the prepared solution a 0.192 mmol concentration of a metal complex (M) and 0.22mmol of compound of Formula II are added to obtain a mixture. The said mixture is then refluxed for 5 hours to obtain a deep red solution. The deep red solution is then evaporated under reduced pressure until the entire methanol, in the solution is distilled off, to obtain a filtrate. A saturated solution of a phosphate salt is added to the filtrate to obtain an orange red solid. The solid product thus obtained is subjected to a second round of filtration and further subjected to a wash cycle with cold diethyl ether and dried under suction. The dried product is then purified by column chromatography using neutral alumina with chloroform/methanol as eluent. The major red band is collected and the complex is precipitated and dried to obtain the compound of Formula I. The yield of the compound of Formula I, as obtained is about 73%. The compound of Formula I, as obtained by the method, as described herein above is then characterised. The compound I, as synthesized herein and as characterized is embedded into a substrate to be used as a probe. The synthesized compound is then characterized to determine structural and emission properties.

CHARACTERISATION OF THE COMPOUND

The compound I shows strong fluorescence property. The synthesized compound is a luminescent metal complex with phenanthroline as the signaling moiety. FIG. 2a shows a plot of emission spectra of compound I in the presence of SLNPV, as observed in water, according to an example of the invention. The compound I showed red fluorescence in water with an

emission maxima (λ_{max}) at 605 nm, upon excitation at 450 nm. Addition of SLNPV from a stock of 10^9 POBs/mL into the aqueous solution of compound I rendered a rapid change in emission color from red to blue.

5 Emission spectra of compound in presence of SLNPV showed a diminution of emission intensity at 605 nm band (~6.5 fold) with concomitant enhancement at 487 nm. FIG. 2b shows a plot of fluorescence titration of compound I in the presence of SLNPV, as observed in water, according to an example of the invention. Titration studies shows a linear concentration-

10 dependent emission quenching with SLNPV over a wide range of concentration of about 0 POBs/mL to about 1.3×10^7 POBs/mL. This can be useful for the determination of SLNPV content in unknown commercial Formulations using the regressive equation, $Y = -11.482 x + 166.682$ ($r^2 = 0.997$). The minimum detectable concentration estimated in this condition is

15 1.68×10^3 POBs/mL.

FIG. 3a shows a plot of emission spectra of the compound I with SLNPV, in different pH ranges, according to an embodiment of the invention. Compound I shows interaction with SLNPV from a pH range of about 4.0 to a pH range of about 10.0. The extent of optical change is higher in the pH

20 range of about 5 to a pH range of about 8. This eventually covers the pH of almost all the agricultural crop extracts known to be affected by SLNPV. FIG. 3b shows a plot of emission spectra of the compound I with SLNPV, in different temperature ranges, according to an embodiment of the invention. Temperature dependent emission studies show that compound I can

25 quantify SLNPV over the temperature range of about 15 °C to about 45°C without any significant error.

To quantify the amount of SLNPV present in the commercial Formulations, the cross-reactivity of compound I towards the other adjuvants present in the mixture is tested. FIG. 4 shows effect of different adjuvants on emission

30 intensity of compound I, according to an embodiment of the invention. No significant alteration in emission signal is observed upon addition of these analytes.

The compound I is further tested for efficacy with active SLNPV Formulations and comparatively ineffective deactivated form which is often found in old, stored Formulations.

- 5 FIG. 5a shows plot of emission spectra of heat treated SLNPV, according to an embodiment of the invention. The heat treated SLNPV solution shows significantly different emission signature in comparison to the fresh one, indicating heat mediated denaturation of the polyhedrin protein layer. Similarly, the heat treated SLNPV solution shows significantly different
10 circular dichroism in comparison to the fresh one. FIG. 5b shows plot of CD spectra of heat treated SLNPV, according to an embodiment of the invention.

- Addition of heat-treated SLNPV to the aqueous solution of compound I shows that the emission change is comparatively to a lower extent. FIG. 6a
15 shows a plot of emission spectra of compound I with heat treated SLNPV, according to an embodiment of the invention. This indicates the preferential affinity of the probe towards the active form SLNPV in water medium. FIG. 6b shows the comparison of compound I interaction with normal and heat treated SLNPV, according to an embodiment of the invention.

- 20 To quantify the specificity of the compound I, the cross-reactivity of compound I is tested with other NPV-biopesticide such as HaNPV.

- FIG. 7a shows a plot of fluorescence titration of compound I with HaNPV at pH 7.0 in water, according to an embodiment of the invention. Emission spectra of compound I is tested at an excitation emission of 450nm, at a
25 concentration range of 10 μ M with HaNPV at a concentration range of about 0 POBs/mL to about 1.3×10^7 POBs/mL. Compound I showed no interaction with HaNPV.

- FIG. 7b shows a plot of change in emission intensity of compound I at concentration range of about 10 μ M, with $\lambda_{ex} = 450$ nm in presence of both
30 HaNPV and SLNPV at pH 7.0 in water, according to an example of the invention.

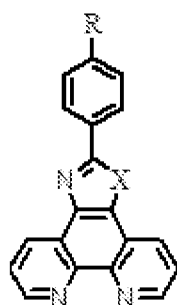
Mode of Interaction with SLNPV

In another embodiment of the invention, the mode of interaction of compound I with SLNPV is tested. Variable temperature emission studies are performed by repeating the titration with SLNPV at temperature range of about 288k to about 318k. FIG. 8 shows changes in thermodynamic parameters of compound I upon interaction with SLNPV, according to an embodiment of the invention.

A negative value of reaction-enthalpy (-2.85 KJ/mol) along with positive entropy change ($\Delta S = 0.0179$ J/mol) suggests that the quenching in emission intensity is predominantly governed by the electrostatic interaction.

In another embodiment of the invention, the zeta potential of SLNPV solution at concentration range of about 0 μ M to about 25 μ M of compound I is tested. FIG. 9 shows a plot of zeta potential of SLNPV solution in different concentration of compound I, according to an embodiment of the invention. In absence of compound I, SLNPV exhibited highly negative zeta potential value owing to its negatively charged polyhedra surface at pH 7.5. Addition of compound I gradually lowered the zeta potential value and became more positive with a zero crossing at ~10 μ M of compound I.

In yet another embodiment of the invention, the mode of interaction of compound I with SLNPV is tested. To study the importance of metal ion center on the interaction process, a compound of Formula II is used in the sensing studies having similar structure to that of compound I devoid of the metal ion center.



FORMULA II

FIG. 10a shows the interaction of SLNPV with compound II, according to an embodiment of the invention. Compound II showed almost no interaction with SLNPV, indicating the relevance of metal ion center in the charge-pair formation. FIG. 10b shows the change in emission intensities of compound I and compound II, with SLNPV at pH 7.0, according to an embodiment of the invention.

FIG. 11 shows a graph depicting change in hydrodynamic diameter of compound I in presence of SLNPV at pH 7.0 in water, according to an example of the invention. The formation of electrostatic association complex is evident from dynamic light scattering measurement, where an increase in hydrodynamic diameter (D_H) is observed upon interaction with SLNPV. However, the circular dichroism studies shows that compound I could destabilize the α -helical conformation of SLNPV during interaction. FIG. 12a shows circular dichroism spectra of SLNPV with compound I, according to an embodiment of the invention. Similarly, FT-IR spectra of SLNPV in presence of compound I shows a shift in stretching frequencies of C=O and amide -NH band in water medium. FIG. 12b shows FT-IR spectra of SLNPV with compound I, according to an embodiment of the invention. The shifts can be corroborated with decrease in the extent of α -helical conformation of SLNPV.

The compound I, as synthesized herein and as characterized is then embedded into a substrate to be used as a probe. The method of embedding the compound I onto the substrate includes constituting the synthesized compound I in an organic medium. Examples of the organic medium include but are not limited to MeOH, DMSO, Acetonitrile, and EtOH. Subsequent to the constitution of the compound I in the organic medium, a substrate suitable for embedding of the compound I is selected. Examples of the substrate include but are not limited to a glass, a quartz, a cellulose matrix wherein the cellulose matrix is synthesized or naturally obtained. The selected substrate is then coated with compound I to obtain a probe. The probe thus obtained is used for detection of SLNPV.

Another embodiment of the invention provides a kit for rapid detection of SLNPV in biopesticidal Formulations. The kit includes a probe, a detection means and an analyser. The probe is obtained by embedding compound I
5 onto a substrate. Examples of the substrate include but are not limited to a glass, a quartz, a cellulose matrix wherein the cellulose matrix is synthesized or naturally obtained. The detection means is a UV source. In one example of the invention, the source is a UV torch. The step of detection includes the determination of the insecticidal efficacy of SLNPV
10 by quantifying the number of occlusion bodies present.

In one embodiment of the invention, the quantification of SLNPV in commercial Formulation is performed prior to spraying of the SLNPV Formulation onto a field having a desired produce. The method involves addition of aqueous dispersion of SLNPV onto the probe. The probe is then
15 placed under UV lamp to observe change in molecular emission of the compound embedded in the substrate. A change in molecular emission from red to blue is observed. The emission change is then verified with the help of an analyser. In one embodiment of the invention, the analyser is a reference chart of emission intensity for known concentrations of SLNPV
20 Formulations. The chart provides reference emission intensities, with respect to a preferred concentration value of the SLNPV Formulation, which is indicative of a minimum threshold value of the concentration required for spraying.

Example: The method as described above is adopted to obtain a probe for
25 measuring the insecticidal efficacy of SLNPV by quantifying the number of occlusion bodies present in the commercial Formulations. Compound I is constituted in 40µl of MeOH to get a final concentration 1 mM. Subsequent to the constitution of the compound I in MeOH, a cellulose based material is selected as the substrate for embedding the compound I. The selected
30 substrate is then coated with the constituted compound I to obtain a probe. The probe thus obtained has strong fluorescence property and is used for the measuring the insecticidal efficacy of the biopesticide, by estimating the

concentration of occlusion bodies present in the commercial Formulations of SLNPV. FIG. 13a shows a plot of emission spectra of the probe with a commercial Formulation of SLNPV, according to an embodiment of the invention. Recovery plot indicates that in a commercial Formulation, quantitative estimation of SLNPV is possible without any significant error. Hence, the probe can act as a quality marker for commercially available SLNPV based biopesticides. FIG. 13b shows a Recovery plot of the probe in presence of commercial Formulation of SLNPV at pH 7.0 in water.

10 The coated paper discs were visualized under a 365 nm UV lamp to confirm the uniform red emission. When SLNPV solution was added onto these precoated paper discs, a clear change in emission color from red to blue was observed under UV lamp. FIG. 14 shows change in the red colour intensity and a graph showing the change in intensity against different concentrations of SLNPV, according to an embodiment of the invention. The extent of change increased with rising concentration of SLNPV in aqueous suspensions of concentration range of about 10^3 POBs/mL to about 10^8 POBs/mL. Further, the relative change in luminescence intensity was quantified by using a readily available image processing software.

15 In yet another embodiment of the invention, the selectivity of the probe towards SLNPV is tested. FIG. 15 shows the change in the intensity of the red color plotted upon addition of different adjuvants of commercial Formulations of SLNPV, according to an embodiment of the invention. There is no significant alteration in the emission spectra upon addition of these analytes. Hence, the probe is suitable for estimation of SLNPV even in commercially available bio-pesticide Formulations. FIG. 16a shows change in the red colour intensity of the probe with fresh and heat treated SLNPV, according to an embodiment of the invention. Heat treated SLNPV solution at a concentration range of about 10^6 POBs/mL is added to the probe to mimic the storage caused deactivated stage. A reduction in the extent of color change is observed in comparison to the fresh one. The difference in emission intensities is quantified using image processing

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software. The test-strips are also employed for the detection of SLNPV in commercial Formulation. FIG. 16b shows change in the red colour intensity of the probe with pure and commercial mixture of SLNPV, according to an embodiment of the invention. A comparison study with pure virus solution indicates that the estimation of SLNPV in commercial Formulation is possible by this method without experiencing any significant loss which is verified by image processing software.

The probe is also tested for change in the red colour intensity upon addition of HaNPV and SLNPV. FIG. 17 shows change in the red colour intensity of the probe upon addition of HaNPV and SLNPV and a plot showing the change in intensity of red colour when plotted against HaNPV and SLNPV, according to an embodiment of the invention. The probe shows sufficiently poor interaction with HaNPV in solution phase. Investigation over a wide range of NPV concentration indicated that the probe can discriminate between these two biopesticide even at a concentration range of about $\sim 10^3$ POBs/mL concentrations.

INDUSTRIAL APPLICATION

The common way to introduce SLNPV in agricultural field is to spray the water-dispersible Formulation of SLNPV using conventional spray equipment. Twenty different types of agricultural crops are selected that are known to be infected by *S. Litura*. Thus for these crops, use of SLNPV as biopesticide is very common for farmers. Emission spectra of compound I is first recorded in presence of these crop extracts to rule out any kind of background interference. Change in emission signal is then monitored upon addition of SLNPV in increasing concentration. FIG. 18a shows emission intensity of compound I with SLNPV in fruits, according to an example of the invention. The dose-dependent gradual decrease in emission intensity at 605 nm shows that the probe can detect SLNPV even in complex mixtures such as crop extracts. FIG. 18b shows emission intensity of compound I with SLNPV in vegetable extracts, according to an example of the invention.

The kit allows specific detection of SLNPV concentration in commercially available biopesticides. The kit is easy-to-use with portable probes that help in measuring insecticidal efficacy of SLNPV by quantifying the number of occlusion bodies present. The use of the probe can reduce the chances of crop loss to a great extent, thereby increasing the economic significance of the crop to the farmers. The on-site detection of SLNPV in commercial Formulations using the probe and the kit, as described herein eliminates the need for skilled personnel for detecting SLNPV, thereby empowering the farmers to be independent and prevent crop loss.

The table below shows minimum detectable concentration of SLNPV in different crop extracts.

Agricultural Crops	Detection Limit (x10 ³ POBs/mL)	Agricultural Crops	Detection Limit (x10 ³ POBs/mL)
Mango	6.03	Cotton	6.80
Orange	6.70	Amaranth	4.95
Apple	6.35	Sorghum	5.20
Strawberry	6.45	Sugar Cane	5.47
Papaya	5.47	Tomato	6.80
Banana	7.38	Egg Plant	6.25
Lettuce	5.82	Beans	5.30
Cabbage	6.10	Broccoli	6.05
Beet Root	5.78	Corn	6.94
Pea Nut	6.72	Ground Nut	6.34

The foregoing description of the invention has been set for merely to illustrate the invention and is not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to person skilled in the art, the invention should be construed to include everything within the scope of the appended claims and equivalents thereof.

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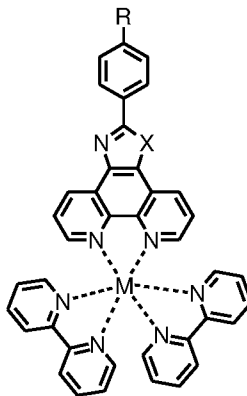
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We claim:

1. A compound for specific detection of SLNPV comprising of Formula I



- 5 The compound of claim 1, wherein M is a tri valent metal ion.
3. The compound of claim 1, wherein the metal ion is selected from a group comprising of Fe, Ru and Os.
4. The compound of claim 1, wherein X is selected from a list comprising
10 of O, S and NH.
5. The compound of claim 1, wherein R is an O-alkyl.
6. A probe for specific detection of SLNPV, the probe comprising of a substrate embedded with the compound of Formula I.
7. The probe of claim 6, wherein the substrate is selected from the group
15 comprising of a glass, a quartz, a cellulose matrix and/or combinations thereof.
8. A kit for specific detection of SLNPV, the kit comprising of the probe as claimed in claims 6-7 and a means for analysing the probe.
9. The kit of claim 8, wherein the means for analyzing the probe comprises
20 of a UV source and a reference chart.
10. The kit of claim 8, wherein the probe containing the compound for specific detection of SLNPV is a fluorescent probe that displays a fluorescence upon shining of the UV source.
11. The kit of claim 8, wherein the fluorescent of the probe is due to the
25 specific binding of SLNPV to the compound of the probe.

12. The kit of claim 8, wherein the reference chart contains a list of color indices corresponding to various concentrations of SLNPV.

13. The kit of claim 8, wherein the reference chart is prepared by recording
5 fluorescence of known concentration of SLNPV.

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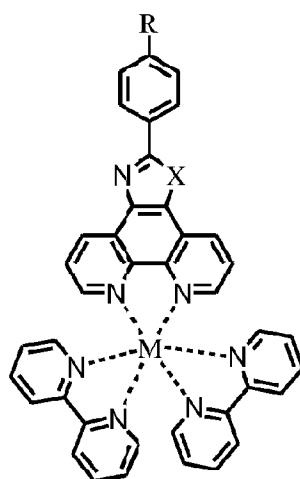
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FORMULA I

FIG. 1

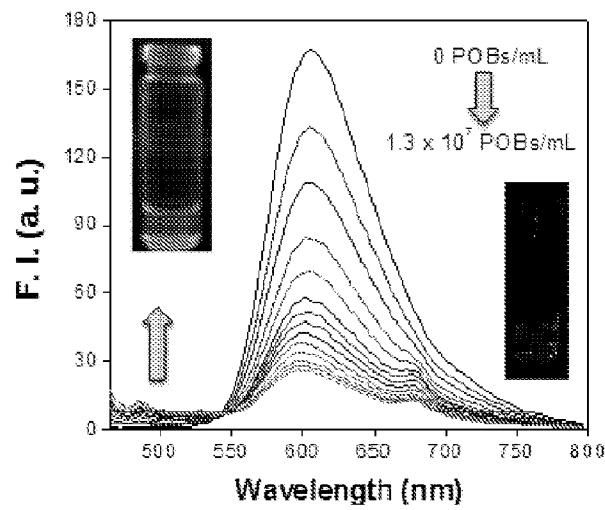


FIG. 2a

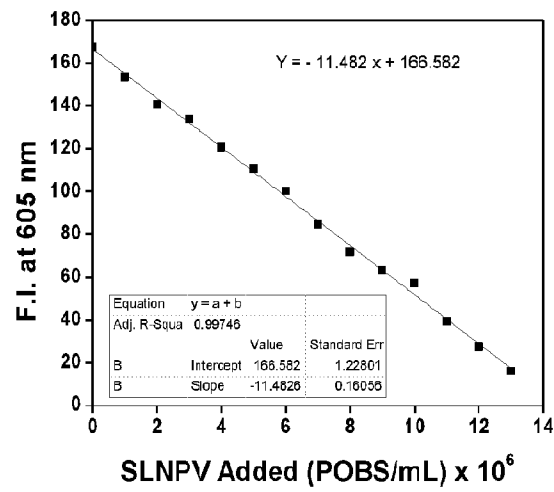


FIG. 2b

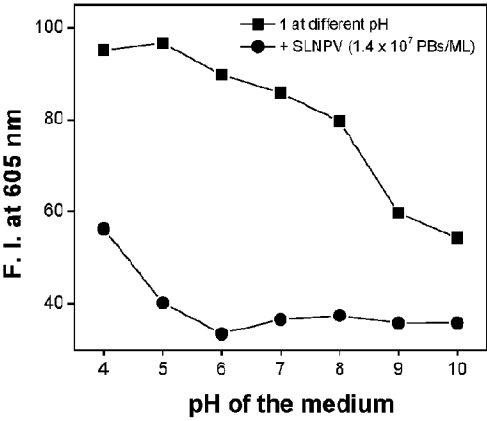


FIG. 3a

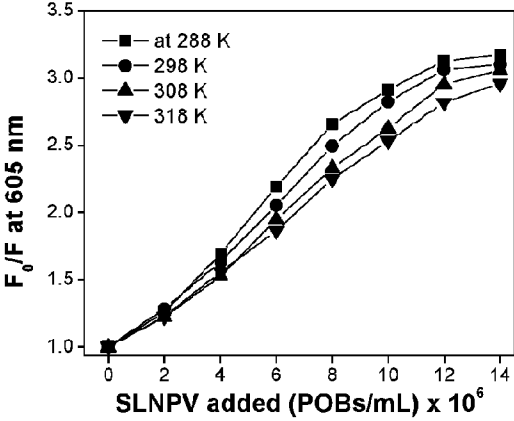


FIG. 3b

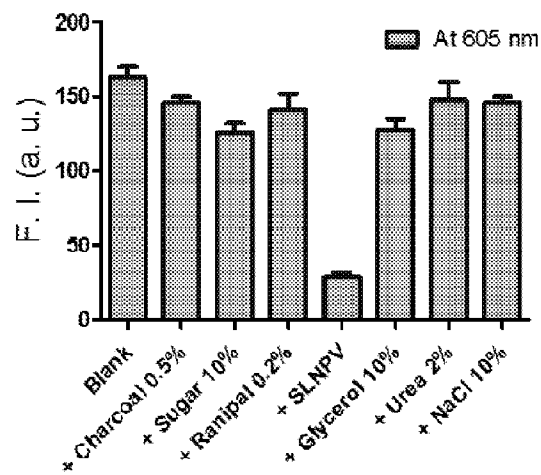


FIG. 4

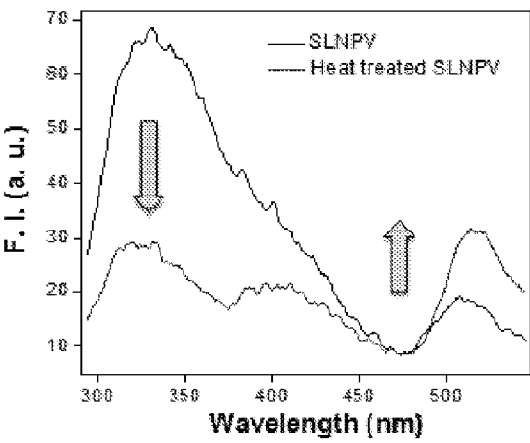


FIG. 5a

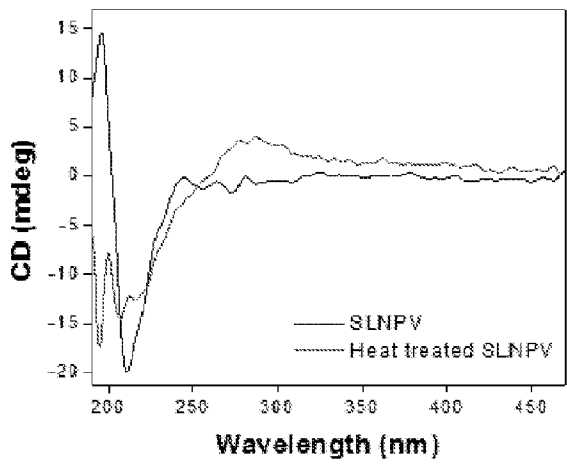


FIG. 5a

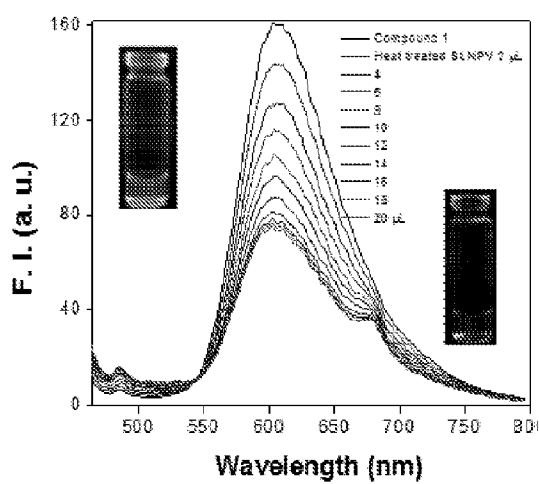


FIG. 6a

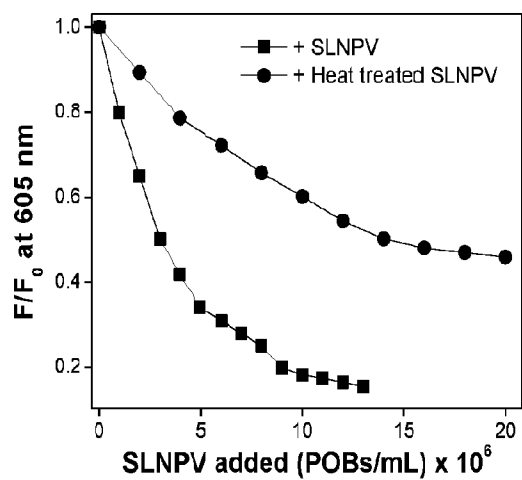


FIG. 6b

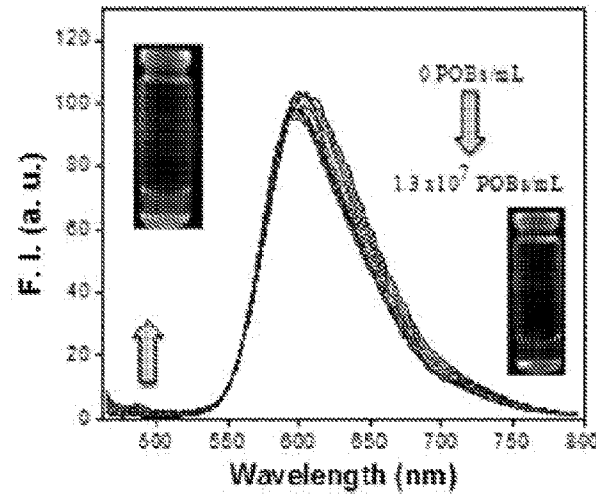


FIG. 7a

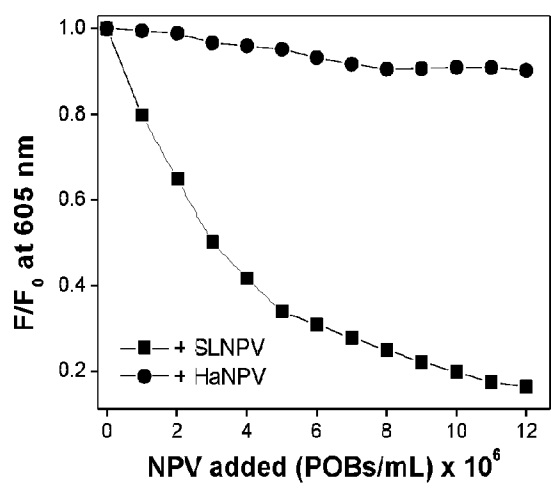


FIG. 7b

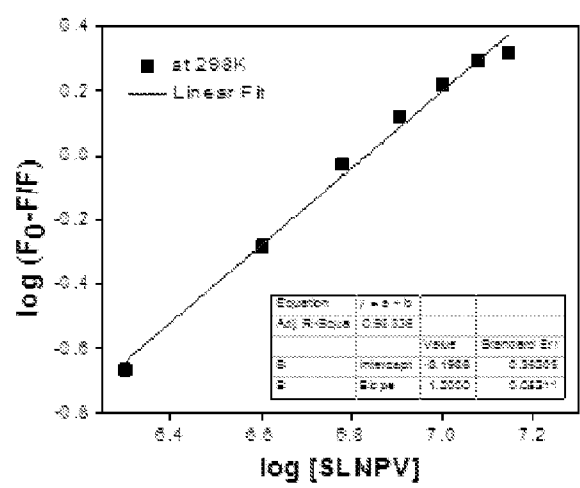


FIG. 8a

Parameter	Value
T	299K
Log K	-8.198
ΔH	-2.85 KJ/ mol
ΔS	0.0179 J/ mol
ΔG	46.78 KJ/mol

FIG. 8b

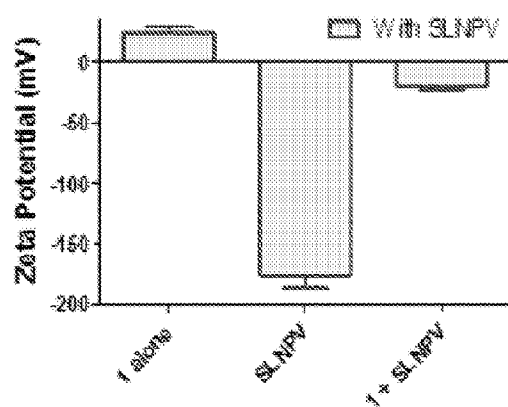


FIG. 9a

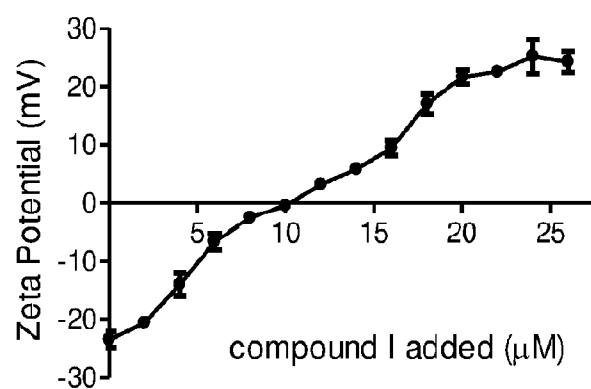


FIG. 9b

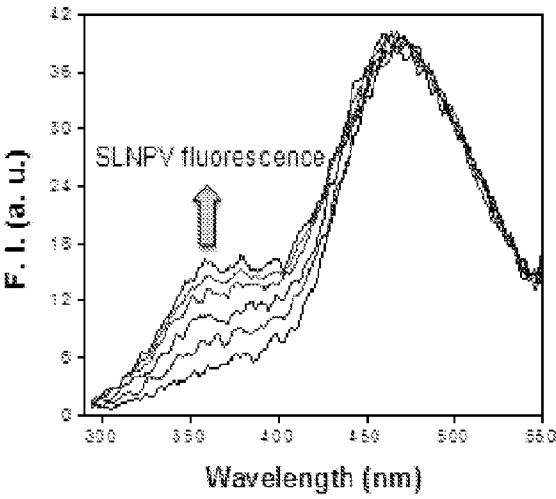


FIG. 10a

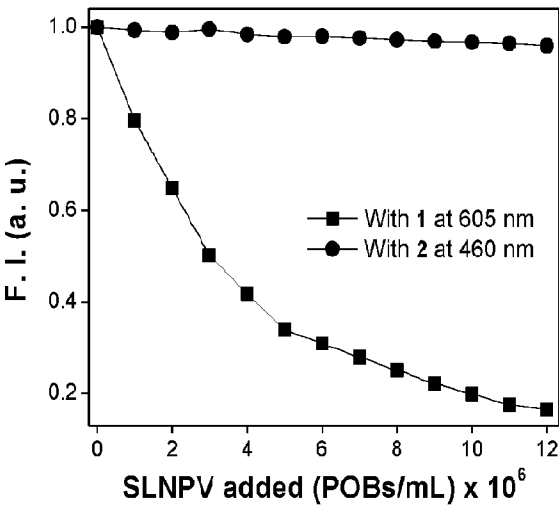


FIG. 10b

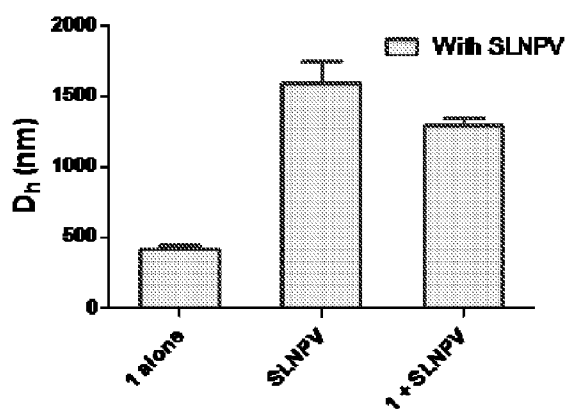


FIG. 11

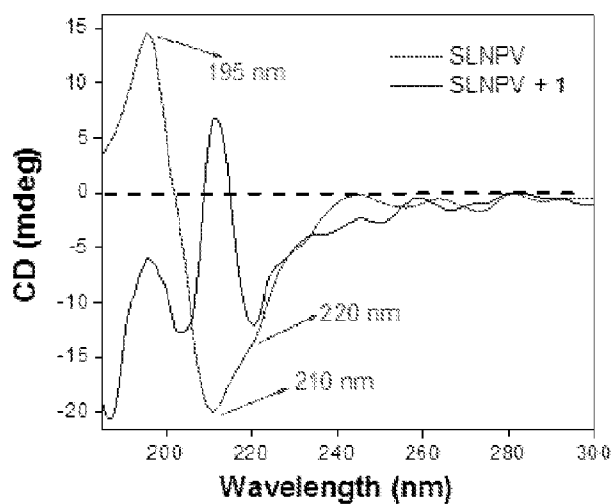


FIG. 12a

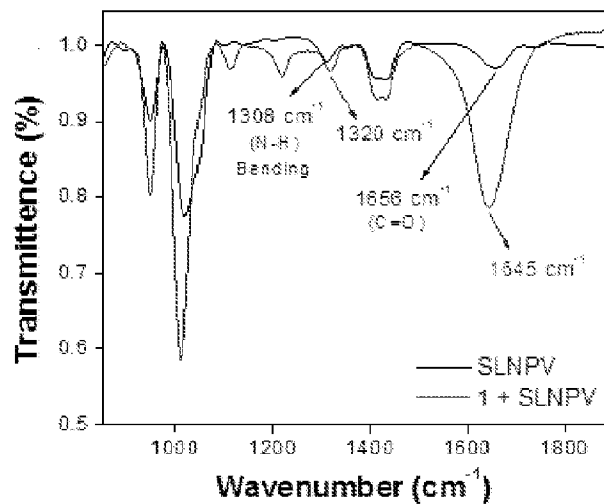


FIG. 12b

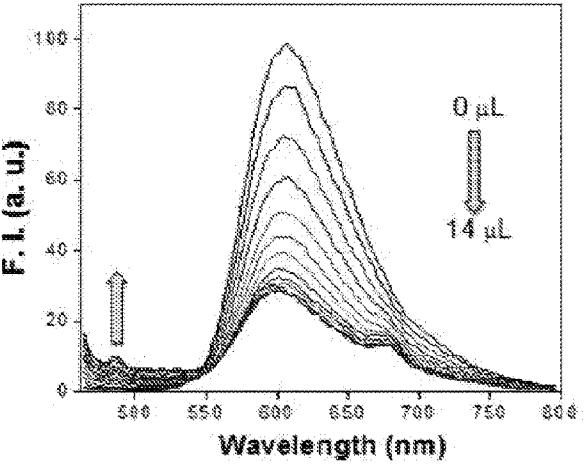


FIG. 13a

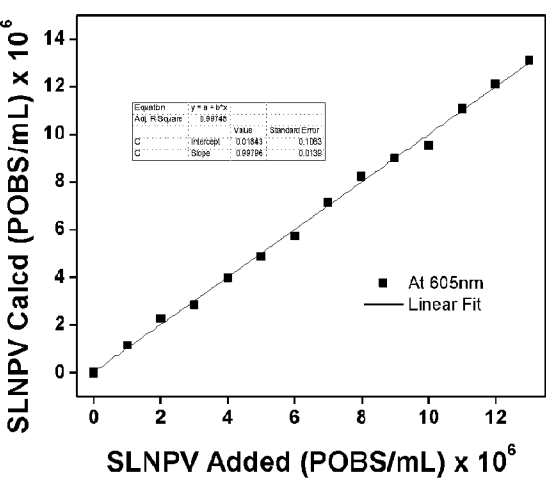


FIG. 13b

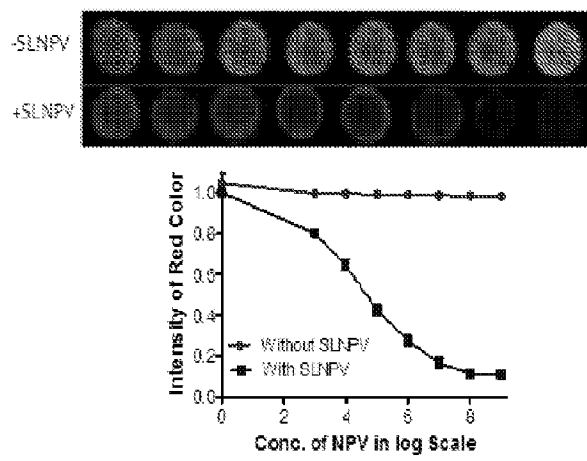


FIG. 14

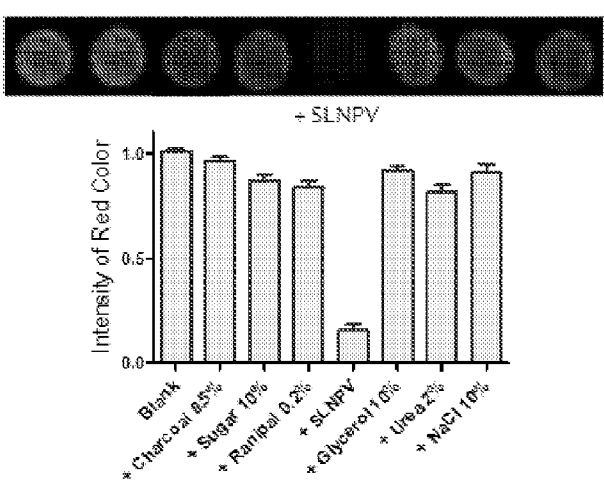


FIG. 15

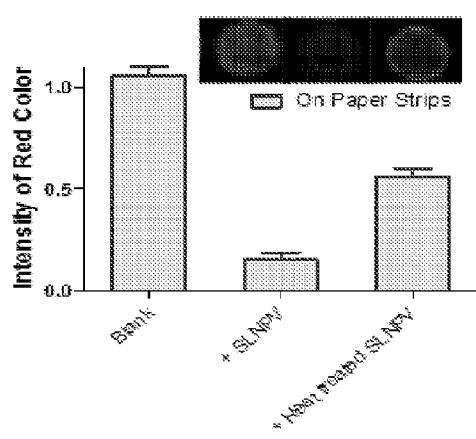


FIG. 16a

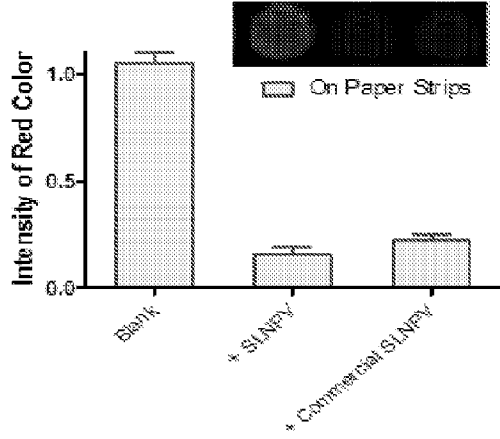


FIG. 16b

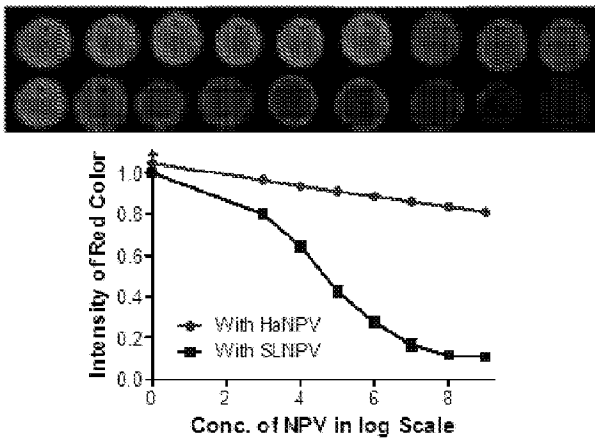


FIG. 17

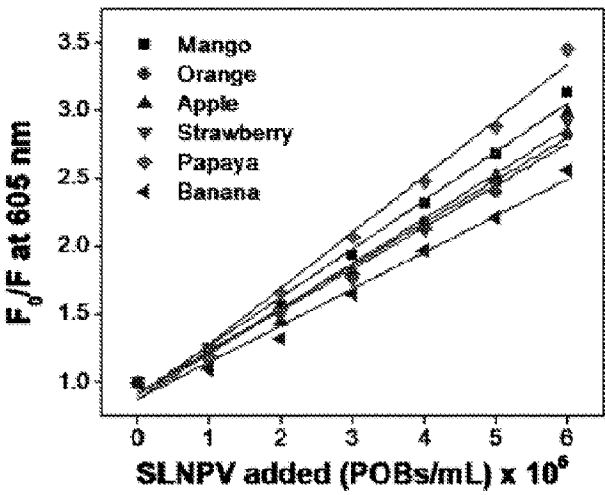


FIG. 18a

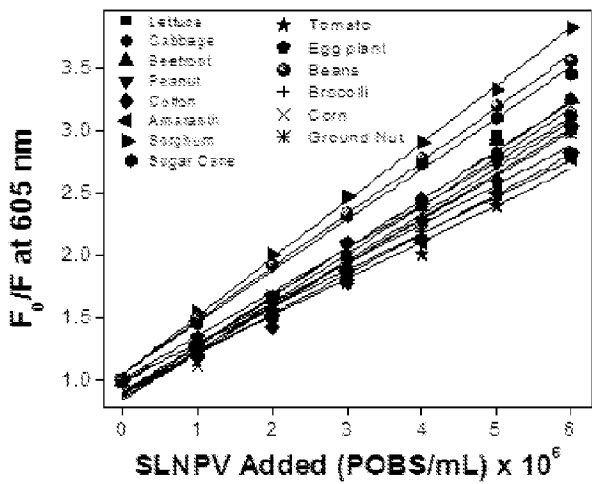


FIG. 18b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2018/050751

A. CLASSIFICATION OF SUBJECT MATTER
C07D515/00, C07D519/00, C07D213/22 Version=2019.01

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

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)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Bis(2,2'-bipyridine)ruthenium(II) complexes with imidazo[4,5-f][1,10]-phenanthroline or 2-phenylimidazo[4,5-f][1,10]phenanthroline, Jian-Zhong Wu et al. J. Chem. Soc., Dalton Trans., 1997, Pages 1395-1401, 1 January 1997 (01-01-1997) page-1395, lines-1-3, 5-8, page-1398	1-13
Y	Bis(4,4'-dimethyl-2,2'-bipyridine) ruthenium (II) Complexes Containing 2-Arylimidazo-[4,5-f][1,10]-phenanthroline:Syntheses Characterization and Third-order Nonlinear Optical Properties, CHAO, Hu et al. DOI-10.1002/cjoc.20020201115, 26 August 2010 (26-08-2010) page-1235-1236, scheme-1	1-13
Y	Ruthenium(II/III) bipyridine complexes incorporating thiol-based imine functions Synthesis, spectroscopic and redox properties, Debadeep Bhattacharyya et al. Polyhedron 18 (1999) 2951-2959, DOI-10.1016/S0277-5387(99)00204-1, 25 October 1999 (25-10-1999) abstract, page-2956, para-3.4	1-5



Further documents are listed in the continuation of Box C.



See patent family annex.

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

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"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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Date of the actual completion of the international search

25-01-2019

Date of mailing of the international search report

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Indian Patent Office
Plot No.32, Sector 14, Dwarka, New Delhi-110075
Facsimile No.

Authorized officer

Debasish Ghosh

Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2018/050751

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DNA Condensation Induced by Ruthenium(II) Polypyridyl Complexes [Ru(bpy) ₂ (PIPSH)] ²⁺ and [Ru(bpy) ₂ (PIPNH)] ²⁺ , Inorg. Chem., Vol. 48, No-11, Bin Sun et al. DOI:10.1021/ic900102r, 4 October 2009 (04-10-2009) page-4637	1-13