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Inventor: GREG E. COLLINS

NOTICE

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WASHINGTON DC 20375

PATENT APPLICATION/TECHNICAL DIGEST PUBLICATION RELEASE REQUEST

FROM: Associate Counsel (Patents) (1008.2)
TO: Associate Counsel (Patents) (1008.2)

Via: (1) Greg Collins(Code 6116)
(2) Division Superintendent (Code 6100)
(3) Head, Classification Management & Control (Code 1221)

SUBJ: Patent Application/Technical Digest entitled: **"SELECTIVE METALS DETERMINATION WITH PHOTOREVERSIBLE REAGENTS"** Request for release for publication.

REF: (a) NRL Instruction 5510.40C
(b) Chapter 6, ONRINST 5870.1C

ENCL: (1) Copy of Patent Application/Technical Digest

1. In accordance with the provision of references (a) and (b), it is hereby requested that the subject Patent Application/Technical Digest be released for publication.

2. It is intended to offer this Patent Application/Technical Digest to the National Technical Information Service, for publication.

3. This request is in connection with Navy Case No. 80,136

11/28/90
(date)

JOHN J. KARASEK
Acting Associate Counsel (Patents)

FIRST ENDORSEMENT Date:

FROM: Greg Collins(Code 6116)
TO: Division Superintendent (Code 6100)

1. It is the opinion of the Inventor(s) that the subject Patent Application/Technical Digest (is) (is not) classified and there is no objection to public release.

Greg E. Collins
Inventor's Signature

SECOND ENDORSEMENT Date:

FROM: Division Superintendent (Code 6100)
TO: Classification Management & Control (Code 1221)

1. Release of Patent Application/Technical Digest (is) ~~(is not)~~ approved.

2. To the best knowledge of this Division, the subject matter of this Patent Application/Technical Digest ~~(has)~~ (has not) been classified.

3. This recommendation takes into account military security, sponsor requirements and other administration considerations and there in no objection to public release.

Division Superintendent

THIRD ENDORSEMENT Date:

FROM: Head, Classification & Control (Code 1221)
TO: Associate Counsel (Patents) (1008.2)

1. This Patent Application/Technical Digest is authorized for public release.

Ina Smallwood
Head, Classification, Management & Control

SELECTIVE METALS DETERMINATION WITH PHOTOREVERSIBLE REAGENTS

Field of the Invention

The present invention relates to a method for differentiating particular metal ions in a complex mixture.

Background of the Invention

Contamination of our waterways and estuaries with metal ions has become a major environmental issue because of the toxic effect these metals have on wildlife, including aquatic plants and fish. Although these metals may be present in small quantities, it is known that they accumulate in aquatic plants and fish, and may be toxic not only to the aquatic plants and fish but also to other wildlife and humans for whom the fish and aquatic plants are a food source. Of particular concern are toxic metals such as lead and mercury, and complexing ions such as copper, zinc and cadmium, which are capable of precipitating protein during the oval stage of fish development.

Both the federal government and individual states have placed strict point source effluent release limitations on the discharge of pollutants into oceans, lakes, and rivers of the United States. Other countries in the global community are following suit. The Uniform National Discharge Standards (UNDS) will result in more stringent nationwide discharge regulations for both metals and organic pollutants. Thus, it will be necessary to monitor discharge water for pollutants in order to prevent discharge of pollutants in violation of waterway regulations. This is of particular importance for water-borne vessels, as bilge water must be monitored to prevent discharge of metal pollutants. For example, in addition to organic pollutants, bilge water analyses of U.S. Navy ships have identified a number of metal pollutants, including lead (0.02-2.90 mg/l), copper (0.32-6.4 mg/l) and zinc (0.08-16.2 mg/l).

A number of fluorochromic and colorimetric dyes have been applied to fiber optic probes to develop sensors for heavy metal or transition metal ions that are of environmental concern. However, these probes generally have suffered from a lack of selectivity and reversibility. The sensors which do exhibit reversibility usually achieve their reversibility through relatively weak interactions between the ligand and the metal, which results in lower sensitivities. Innovative efforts to overcome these problems have typically relied upon incorporating both the complexation or recognition site, and a light-or-potential induced rejection mechanism into a single sensing molecule. Examples in the literature which have had varying levels of success include: a ferrocene linked crown ether, a photochromic azobenzene-"butterfly", a viologen cyclophane, chromenes, spirooxazines, and spiropyrans.

Additionally, current detection methods for metal ions often require bulky or sensitive instrumentation, making measurements in the field impractical. As a result, environmental samples must currently be transported to an analytical laboratory, resulting in significant time delays and a greater potential for contamination or sample degradation during storage. It is known that storage can alter the metal-ion concentration of a sample through adsorption of the metal on the surface of the container, or by the growth of microorganisms that can alter the speciation of metal ions in the sample.

Kuhn et al., in U.S. Patent No. 5,648,270, disclose flourescent indicators for identifying metal cations. These indicators are fluorophore conjugates of pyridyl-based metal ion chelators. However, this method of detection and assay requires monitoring changes in the indicator's fluorescent properties, *e.g.*, quantum yield, lifetime, polarization, or wavelength, upon binding the target ions by detecting the resultant light emissions. This requires the use of visual inspection, CCD cameras, video cameras, photographic film, or the use of current instrumentation such as a laser scanning device, fluorometers, photodiodes, quantum counters, epifluorescence, microscopes, scanning microscopes, flow cytometers, fluorescence microliter plate readers, or by amplifying the signal with a photomultiplier tube.

Other methods for testing for metal ions in water are illustrated in Miyashita, U.S. Patent No. 5,288,592; Sonte, U.S. Patent No. 5,912,180; and Byrne, U.S. Patent No. 4,092,117.

Summary of the Invention

It is an object of the present invention to overcome the aforesaid deficiencies in the prior art.

It is another object of the present invention to detect metal ion pollutants at very small concentrations.

It is another object of the present invention to provide a method for detecting metal ions using a photoreversible metal binding ligand in combination with partial least squares discriminant analysis.

It is another object of the present invention to provide a reversible method for detecting a plurality of metal ions.

According to the present invention, a photoreversible metal binding ligand is used to identify metal ions in solution. In particular, spiropyrans modified to incorporate a metal ion complexation site such as, 8-hydroxyquinoline, onto the backbone of the spiropyran, are used as the photoreversible metal ion sensing reagents. The photoreversible ejection of complexed metal ions is based upon the geometric reconfiguration resulting from photoisomerization of the photoreversible compound.

Spiropyrans are photochromic materials which can photoisomerize between an "open" and a "closed" form based upon their exposure to UV or visible light, as shown in Figure 1. When the spiropyran is modified to incorporate a metal ion complexation site onto its backbone, as in nitroquinolinospiropyranindoline, NQSP, it becomes possible for the photoreversible ejection of complexed metal ions based upon the geometric reconfiguration resulting from the spiropyran-merocyanine photoisomerization, as shown in Figure 1.

The spiropyrans can be substituted with any substituent that complexes metal ions as long as the photoisomerization properties are not inhibited. The important characteristic of these compounds is their photoreversibility and their ability to photoreversibly eject complexed metal ions from the molecule.

The characteristics required for the spropyrians are that they complex photoreversibly with metal ions and exhibit spectral changes. Preferably, the spectral changes are in the visible wavelengths.

In addition to bearing photo reversibility of metal ion complexation, the NQSP compounds also benefit from spectral shifts in absorbance that depend upon the type of metal ion. Because of the complexity of the mixture ultimately envisioned for analysis by this type of probe molecule in waste water samples, and because of the different regulatory rules applicable to each respective metal ion, selective determination of metal ion type and concentration is critical to preventing false alarms. Despite the shifts in λl_{max} seen for complexation of different divalent metal ions by the NQSP compounds, there remains significant overlap of the spectra for multiple component samples, necessitating the use of chemometrics for unraveling the selective contribution to the spectra from each complexed metal ion.

Other researchers have successfully applied chemometric techniques to metal ion determinations using single metal chelating ligands, including 8-hydroxyquinoline-5-sulfonic acid (HQS), PAR, 1,5-bis-(d-2-pyridylmethylene)thiocarbonohydrazide (DPTH), tribromochlorophosphonazo, and 4-nitrophenylfluorone. However, the present invention is the first application of chemometric methodologies to the selective determination of metal ions, in a photoreversible complexation ligand.

The present invention is particularly well suited to detection of divalent transition metal ions, divalent alkaline earth metal ions and triply charged metal ions such as Al(III). The spiropyrams do not react with singly charged alkali metal ions.

Discriminant analysis models have been built using partial least-squares discriminant (PLSD) analysis to different among Zn^{2+}, Co^{2+}, Hg^{2+}, Cu^{2+}, Cd^{2+}, and Ni^{2+}. PLSD is an adaptation of the well-known partial least-squares regression (PLSR) calibration technique for application to classification problems. PLSD models are particularly well suited to applications involving a large number of highly overlapping broad analyte signatures such as those found in UV/visible, fluorescence, and near-infrared spectroscopy.

Brief Description of the Drawings

Figure 1 shows the photoreversible equilibria and metal complexation of nitroquinolinospiropyranindoline (NQSP) with a divalent metal ion.

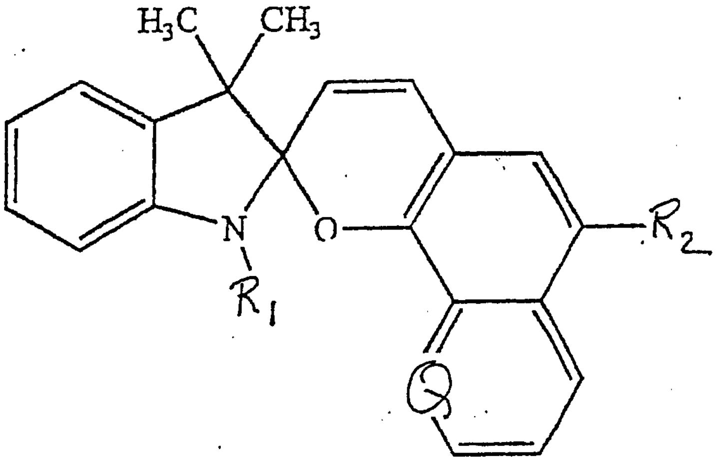
Figure 2 shows a photoreversibility of NQSP (right) and $[Hg\text{-}NQSP]^{2+}$(left).

Figure 3 shows a visible spectra of nitroquinolinospiropyranindoline (NQSP) and its complexes with Hg^{2+}, Cd_{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} in dicapryl phthalate.

Figure 4 shows the absorption spectra of 25 µM nitroquinolinospiropyranindoline (NQSP) in dicapryl phthalate containing zero-25 µM Zn^{2+}.

Detailed Description of the Invention

The substituted spiropyrans that can be used in the present invention are preferably of the formula



wherein Q is a Lewis base such as oxygen or sulfur;

R_1 is C_1-C_{10} straight or branched chain alkyl;

R_2 is a strong electron acceptor such as NO_2; NR_3R_4, wherein R_3 and R_4 are each hydrogen or C_1-C_6 alkyl; C_6-C_{30} straight or branched chain alkyl or substituted alkyl which can have a chemical attachment group such as thiol carboxylic acid, or $SiCl_3$ at the end to allow covalent attachment to a substrate surface.

Additionally, R_1 can optionally be substituted with an attachment group such as carboxylic acid, thiol, or $SiCl_3$ for anchoring the molecule to a substrate.

Photochromic Behavior of NQSP in Dicapryl Phthalate

Typical photochromic behavior was observed for NQSP in dicapryl phthalate. Irradiation of compound **2** of Figure 1, the "open" form, with visible light from a 150 W tungsten-halogen lamp caused the solution to change from purple to colorless in 28 seconds, which indicated photoisomerization and the formation of compound **1** of Figure 1, the "closed" form (*cf*. Figure 2). Conversion from **1** to **2** began immediately after visible irradiation from the flood lamp had ceased, with the purple color of **2** returning completely after 84 seconds.

Similar behavior was observed for the photoisomerization of **3** to **1**. The red color of **3**, for example, changed to nearly colorless following visible light exposure from a flood lamp for 15 seconds. This transition indicates photoisomerization of **3** to **1**, and is a strong indicator that the complexed Hg^{2+} ions have been ejected into the solution (with approximately 93% ejection efficiency). After visible irradiation ceased, the red color of the $[Hg\text{-}NQSP]^{2+}$ complex returned after two minutes due to recomplexation of the free Hg^{2+} ions in solution by NQSP.

Effect of Divalent Metal Ions

The absorbance spectrum of NQSP in dicapryl phthalate has a λ_{max} at 580 nm, as shown in Figure 3. The addition of divalent metal ions to NQSP in dicapryl phthalate produces solutions of various colors with overlapping absorption spectra. For example, an orange color was observed for the Zn^{2+}-NQSP complex (λ_{max} = 524 nm), while a wine-red color was observed for the Hg^{2+}-NQSP complex (λ_{max} = 550 nm). Spectra for each of the NQSP-divalent metal ion complexes in dicapryl phthalate studied here are shown in Figure 3. Spectral shifts seen in this plasticiser were very similar to those obtained in organic solvents such as THF and acetonitrile, and each of the different metal complexes exhibited photoreversibility following irradiation with visible light from a flood lamp. Additional spectral properties of NQSP and NQSP-metal ion complexes are summarized in Table 1. As expected, increasing the concentration of metal ions in solution results in a linear increase in the absorbance of the hypsochromic shifted peak, as shown in Figure 4.

Table 1. Absorption Properties of NQSP and NQSP-Metal Complexes

Metal	*Molar Absorptivity 10^3* ($M^{-1}cm^{-1}$)	λ_{max} (nm)	$\Delta\lambda_{max}$ (nm)[a]
None	8	580	–
Cu^{2+}	16	519	61
Zn^{2+}	11	524	56
Hg^{2+}	19	550	30
Ni^{2+}	9	533	47
Co^{2+}	17	518	62
Cd^{2+}	18	538	42

[a] $\Delta\lambda_{max}$(NQSP with no metal) - λ_{max}(NQSP with metal)

Single Component Spectra

In PLSD, a y variable is simply an indicator (dummy) variable composed of either +1 or −1 to indicate the presence or absence of a particular analyte. The inputs to PLSD are the spectra (n x m, where n is the number of spectra and m is the number of wavelengths) and the y values (n x 1). PLSD analysis uncovers the underlying factors or latent variable (h) that explain the covariance between the spectra and y. The product is a multivariate classification model of the form,

$$y_i = b_1 x_{i,1} + \dots + b_m x_{i,m} \qquad (1)$$

Where y_i indicates the presence (+1) or absence (-1) of the metal in spectrum i, the x_i terms are the spectra, while the b terms are the multivariate discriminant coefficients. Because information about both the metal of interest and the interfering species are encoded in the discriminant coefficients, the model accounts for interfering metal ions.

This feature enables PLSD to produce classification models for an analyte even the presence of extreme spectral overlap. A key parameter in the development of a PLSD is the number of latent variables or PLS factors. In this work, the number of PLS factors was chosen to be the value that results in the minimum classification error in the training subset.

Metal identification is performed by computing the scalar inner product of the spectrum and the PLS discriminant coefficients for that metal (equation 1). If the predicted y value is greater than zero, then that metal is considered to be present. For the purposes of the present invention, a separate PLSD was computed for each of the six divalent metal ions using just the spectra in the training subset. Prediction spectra were withheld from training and only used to validate the classification performance of the discriminate.

Initial experiments focused on determining whether the discriminant analysis could distinguish among the spectra of the six different metals tested. Table 2 gives a breakdown of the 28 spectra in the single component data set. Seven spectra were randomly selected as the holdout prediction (validation) set. The prediction spectra were restricted to include only those spectra that were not measured at either the highest or the lowest concentration for a particular metal. The prediction subset was also constrained to include only one or two spectra from each metal. Using the random number generator from MATLAB, seven spectra (7/28, or 25%) were randomly selected (A3, A8, A12, A13, A17, A23, A26). The remaining spectra were placed in the training subset.

Table 2. Single Component Metal Samples

Metal	Samples	Concentrations (10^{-6}M)
Cu^{2+}	A1, A2, A3, A4, A5	5, 10, 15, 20, 25
Zn^{2+}	A6, A7, A8, A9, A10	5, 10, 15, 10, 25
Hg^{2+}	A11, A12, A13, A14, A15	5, 10, 15, 20, 25
Ni^{2+}	A16, A17, A18, A19	5, 10, 15, 20
Co^{2+}	A20, A21, A22, A23, A24	1.5, 2.5, 5, 7.5, 10
Cd^{2+}	A25, A26, A27, A28	5, 10, 20, 25

A PLS discriminant for each metal was trained using the 21 calibration spectra. Investigations were performed to determine the optimal spectral range and number of PLS factors. For this data set, the spectral range was not critical. In all cases, perfect training (126/126 = 100%, where 21 calibration samples x 6 metal ions = 126) and prediction results (42/42 = 100%, where 7 production samples x 6 metal ions = 42) could be obtained using the entire spectrum (400-700nm). These results indicate that despite the strong spectral overlap, PLS discriminants can easily differentiate among the six different metals studied.

Table 3. Two-Component Mixture Selectivities

	Cu^{2+}	Zn^{2+}	Hg^{2+}	Ni^{2+}	Co^{2+}	Cd^{2+}
Cu^{2+}	–	0.14	0.76	0.17	0.17	0.50
Zn^{2+}	–	–	0.81	0.29	0.21	0.56
Hg^{2+}	–	–	–	0.74	0.84	0.36
Ni^{2+}	–	–	–	–	0.26	0.46
Co^{2+}	–	–	–	–	–	0.60
Cd^{2+}	–	–	–	–	–	–

Thus, additional metals can be added to the discriminant pool without a problem, making it possible to analyze for a plurality of metal with one reagent.

Two Component Spectra

Based upon the success of the first phase and the large selectively (SEL) values determined from net analyte signal (NAS), analysis (see Table 3) tests were conducted to selectively identify metals present within a mixture. An additional data set was collected using the same experimental protocol used above. The metals chosen for mixture analysis were selected to endure a range of spectral overlaps (*i.e.*, SEL). One easy mixture, Cu-Hg, and two challenging mixtures, Zn-Ni and Zn-Co, were selected. This data set consisted of spectra from a total of 19 binary mixtures of metals and is summarized in Table 4.

Table 4. Two Component Metal Samples

Sample #	*Metals*		*Concentrations (10^{-6}M)*	
T1	Zn^{2+}	Ni^{2+}	20	10
T2	Zn^{2+}	Ni^{2+}	10	10
T3	Zn^{2+}	Ni^{2+}	15	10
T4	Cu^{2+}	Hg^{2+}	10	10
T5	Cu^{2+}	Hg^{2+}	15	10
T6	Cu^{2+}	Hg^{2+}	20	10
T7	Zn^{2+}	Ni^{2+}	15	10
T8	Zn^{2+}	Ni^{2+}	15	5
T9	Zn^{2+}	Ni^{2+}	15	3
T10	Zn^{2+}	Ni^{2+}	7.5	5
T11	Zn^{2+}	Ni^{2+}	7	7
T12	Zn^{2+}	Ni^{2+}	4	10
T13	Zn^{2+}	Ni^{2+}	4.5	3
T14	Zn^{2+}	Co^{2+}	5	4
T15	Zn^{2+}	Co^{2+}	7.5	4
T16	Zn^{2+}	Co^{2+}	10	4
T17	Zn^{2+}	Ni^{2+}	3	3
T18	Zn^{2+}	Ni^{2+}	1.5	3
T19	Zn^{2+}	Ni^{2+}	4.5	3

To make the test as challenging as possible, the 28 single-component spectra from phase 1 (Table 2) were used as the training set (A1-A28), while the 19 mixture spectra (T1-T19) were used only for prediction. A single PLS discriminant was trained for each metal. Studies were conducted to determine the optimal spectral range and number of latent variables for each discriminant. In all cases, the smallest number of latent variables required to achieve perfect training classification performance was selected as optimal. There were no missed detections or false alarms in the training set.

Table 5 presents a summary of the training and prediction results. The prediction classification performance for PLSD was better than expected. In 16 of the 19 production samples, both metals were correctly identified. In samples T6, T12, and T17, only one of the two metals was correctly identified (3 missed detections), and there were no false detections. Considering all possible decisions (19 samples x 6 metal ions = 114), a prediction classification performance of 111/114 = 97.5% can be reported. This is especially remarkable in light of the fact that the training set consisted of single-component spectra only.

Table 5. Pattern Recognition Results from the Two-Component Mixtures Dataset

Metal	***Spectral Range (nm)***	***Training***	***Prediction***
Cu	400-558	28/28	19/19
Zn	430-489	28/28	18/19
Hg	400-699	28/28	18/19
Ni	517-630	28/28	18/19
Co	459-630	28/28	19/19
Cd	400-558	28/28	19/19

The advantages of the sensing methodology of the present invention relate to the combination of sensitivity, selectivity, and reversibility. There are numerous dyes that incorporate one or even two of these sensing characteristics, *e.g.*, sensitivity and selectivity, however, none has combined all three characteristics into a single probe design for monitoring metal ion pollutants. Without a reversible metal ion sensing probe, the irreversible complexation of metal ions by the sensing dye results in the formation of one-time use sensors which are costly and inconvenient due to their limited lifetimes. The selectivity obtained through using chemometric modeling permits analysis of more complex metal ion containing matrices known to contain more than one interfering ion. Finally, the high molar absorptivity of NQSP makes ppb sensing of metal ions feasible using inexpensive, visible wavelength transmitting fiber optics and associated instrumentation.

For remote observation, the indicator can be incorporated as part of a fiber optic probe. In this embodiment of the invention, the indicator is covalently attached to a fiber optic probe material, typically glass or functionalized glass (*e.g.*, aminopropyl glass), or the indicator is attached to the fiber optic probe via an intermediate polymer such as polyacrylamide. The indicator solution may alternatively be incorporated non-covalently within a fiber optic probe, or separated from the sample by a semi-permeable membrane, as long as there is a means for the target metal to contact the indicator solution.

Alternatively, the photoreversible dye may be incorporated in an optical waveguide device or a transmission spectrometer, or coupled to a substrate by covalent or other binding. This coupling is effected by conventional methods which are well known to those skilled in the art.

The photoreversible dye may be supported in any of a number of plasticizers, nonaqueous solvent, solgels, or other containment media which are sufficiently nonpolar to support the metal ion ejection event. Moreover, a membrane can be used both to support the entrapment media and to permit the diffusion of metal ions.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept. Therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means and materials for carrying our various disclosed functions make take a variety of alternative forms without departing from the invention. Thus, the expressions "means to..." and "means for..." as may be found in the specification above and/or in the claims below, followed by a functional statement, are intended to define and cover whatever structural, physical, chemical, or electrical element or structures which may now or in the future exist for carrying out the recited function, whether or not precisely equivalent to the embodiment or embodiments disclosed in the specification above; and it is intended that such expressions be given their broadest interpretation.

ABSTRACT OF THE DISCLOSURE

Photoreversible compounds are used as metal ion sensing reagents. Particularly, spiropyrans modified to incorporate a metal ion complexation site such as, 8-hydroxyquinoline, onto the backbone of the spiropyran, are used as the photoreversible metal ion sensing reagents. Partial least-squares discriminant analysis is used to differentiate among the metal ions in a solution.

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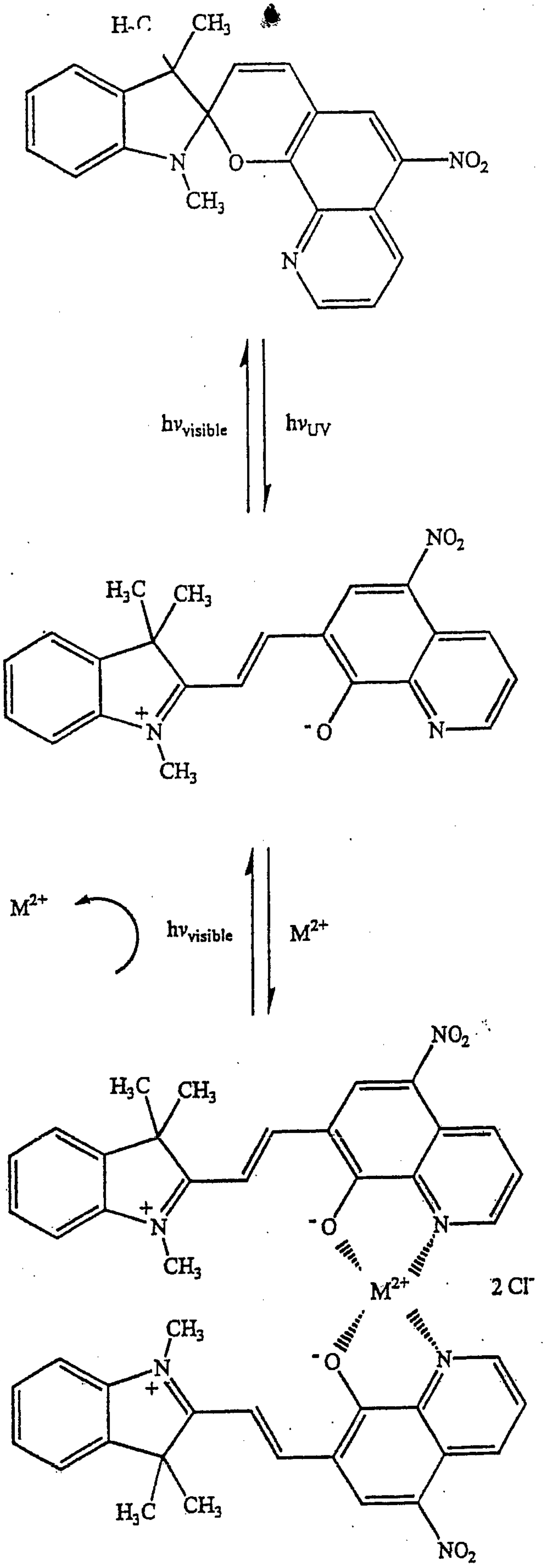

1, "Closed"

2, "Open"

3, "Complexed"

FIGURE 1

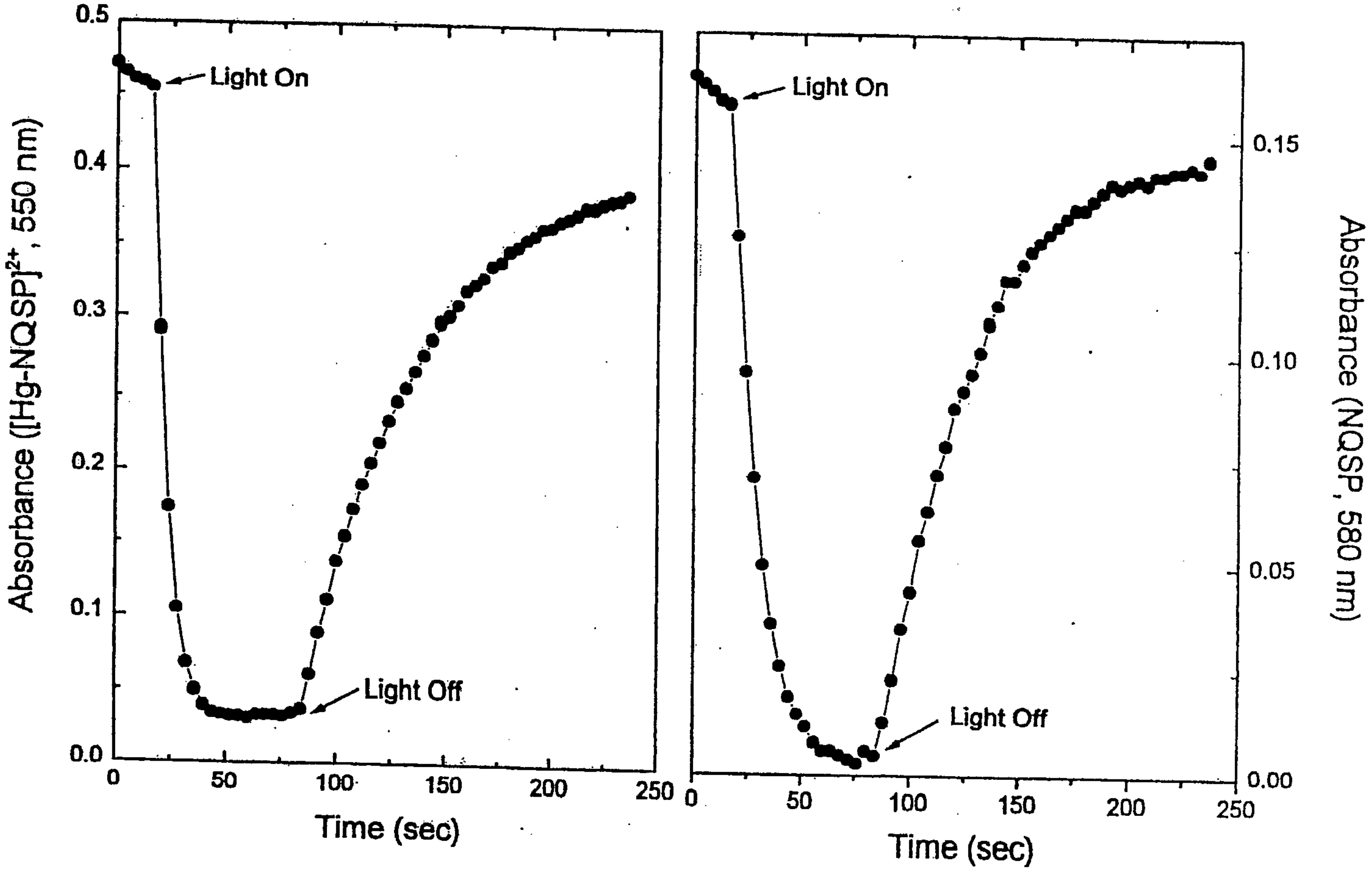
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance ([Hg-NQSP]$^{2+}$, 550 nm)
Light On
Light Off
0
50
100
150
200
250
Time (sec)
Light On
Light Off
0
50
100
150
200
250
Time (sec)
0.15
0.10
0.05
0.00
Absorbance (NQSP, 580 nm)

FIGURE 2

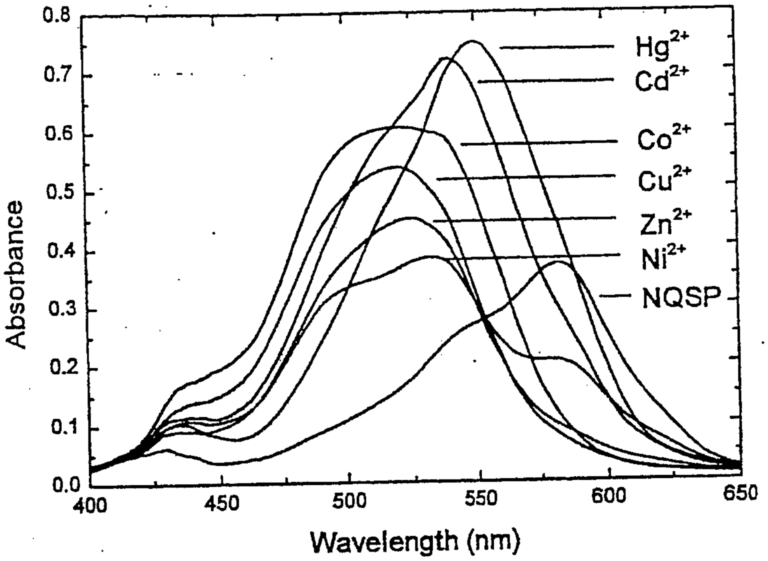

FIGURE 3

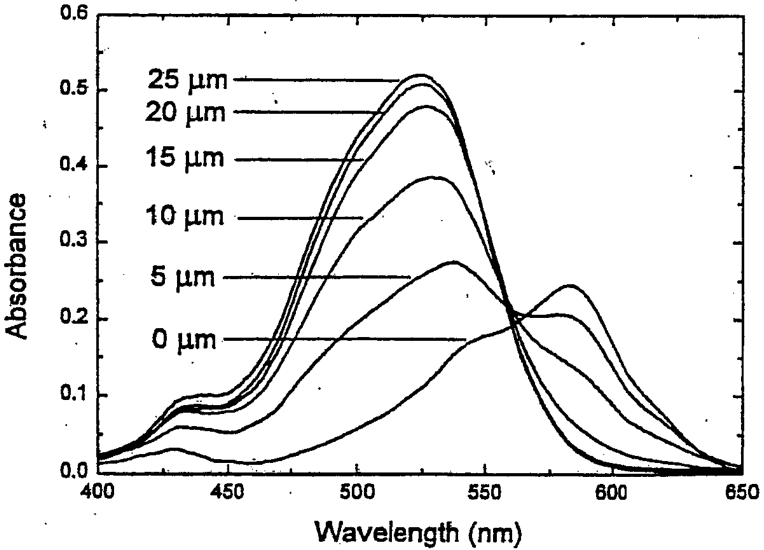

FIGURE 4