Rhodamine and BODIPY chemodosimeters and chemosensors for the detection of Hg 2+ , based on fluorescence enhancement effects

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Rhodamine and BODIPY chemodosimeters and chemosensors for the detection of Hg^{2+}, based on fluorescence enhancement effects

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Fluorescent sensors for Hg^{2+} are demonstrating their potential in a variety of fields such as environmental and biological applications. The review focuses on the recent development of rhodamine derivatives in which the spirolactam (non-fluorescent) to ring-opened amide (fluorescent) process was utilized and on the development of BODIPY derivatives in which the photoinduced electron transfer (PET) process was utilized. New trends in the immobilization of the molecular probes on solid supports, as polymers and/or nanostructures, have been emphasized. The different recognition mechanisms used for the signal responses have been analyzed. The spectroscopic properties, reaction media, analytical parameters, interferences by other ions and practical applications have been summarized.

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1 Introduction

There is great interest in the development of good sensors for the detection of heavy metal ions because, although some have vital and beneficial effects, the toxicity of others is of particular concern. It is a fact that Hg^{2+}, Cd^{2+}, Pb^{2+} and As^{3+} are among the most toxic ions known that lack any vital or beneficial effects and that accumulation of these over time in the bodies of humans and animals can lead to serious illnesses. Specifically, Hg^{2+} is considered as one of the most hazardous environmental

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Arsenio Muñoz de la Peña received his PhD (1981) degree at the University of Extremadura, Badajoz, Spain. He held post-doctoral positions at the Department of Medicinal Chemistry, University of Florida, and Department of Chemistry, University of Emory, and maintained a long-term collaboration with the Universities of Rosario and Litoral (Argentina). He is full Professor and Head of the Department of Analytical Chemistry at the University of Extremadura. He is author of more than 170 scientific articles, reviews and book chapters. His research interest includes analytical applications of luminescence, multi-way multivariate calibration, and the development of luminescence chemosensors.

contaminants.[1–3] It is widely distributed in air, water, and soil[4,5] through different processes such as volcanic emissions, mining, solid waste incineration, and the combustion of fossil fuels.[6–8]

Of particular concern is the ability of some anaerobic organisms to transform the elemental and inorganic forms of mercury into methylmercury, allowing its entrance in the food chain through bioaccumulation in edible animals, and subsequent introduction in the human body.[9–12] Besides, mercury ions can easily pass through biological membranes[13] and show a high affinity for thiol groups in proteins.[14,15]

It is a considerably dangerous metal to human life and ecology even at low concentrations. Among the serious health consequences due to the presence of Hg^{2+} in the human body are prenatal brain damage, DNA damage, various cognitive and motion disorders, Minamata disease, myocardial infarction, some kinds of autism and damage of the brain, kidneys, central nervous system, immune system and endocrine system.[13–17]

The usual methods for determination of mercury are atomic absorption spectroscopy,[18–20] inductively coupled plasma mass spectrometry (ICP-MS),[21–23] capillary electrophoresis–ICP-MS[24] and high performance liquid chromatography–ICP-MS.[25–27]

Taking into account the fact that these techniques demand expensive and very complicated sample pretreatment and instrumentation, the development of optical probes able to translate molecular recognition into tangible optical signals is highly demanded. Moreover, the synthesis and design of optical sensors are focused on the capability of detecting mercury at a low cost with high sensitivity and selectivity.[9,28] Noticeably, there is a great variety of chemosensors and chemodosimeters capable of providing useful information about the appearance of mercury in different matrices.

In this context, fluorescent molecular sensors are becoming more and more important in mercury detection due to their easy use, low cost and high efficiency. Generally, Hg^{2+} ions are known to produce fluorescence quenching when binding to fluorophore molecules *via* the spin–orbit coupling effect. In consequence, the turn-off is the usual response upon binding in most instances, and the sensors with fluorescence enhancement (turn-on response) are still rare. However, fluorescent probes showing fluorescence enhancement on binding to Hg^{2+} are preferred rather than quenching for the design of a metal-ion selective fluorescent sensor, as due to the ubiquitous nature of fluorescence quenching, their sensitivity and practical utility are to some extent reduced.

Therefore, development of fluorescent turn-on type response molecular probes for monitoring the level of Hg^{2+} in environmental and biological samples is of current interest.[29]

2 Fluorescent chemodosimeters and chemosensors

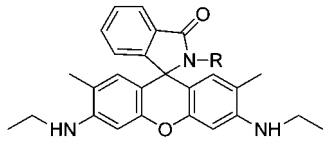
Chemodosimeters are devices, molecule-sized or larger, based on receptors, to achieve analyte recognition with concomitant irreversible transduction of a human-observable signal.[30] A chemodosimeter allows analyte detection through a highly selective and usually irreversible chemical reaction between the

Alejandro Machuca was born in Badajoz, Spain, in 1988. He received his B.Sc. (2011) and M.Sc. (2012) from the University of Extremadura, and is performing pre-doctoral research at the Department of Analytical Chemistry at the University of Extremadura. His research interest is related to the development of luminescence chemosensors and chemodosimeters for environmental analysis.

Héctor Goicoechea was born in Santa Fe, Argentina, in 1961. He received his PhD (2000) from the National University of Rosario. After completing postdoctoral research at Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, USA, he joined the faculty at National University of Litoral, Santa Fe, in 2004, where he is currently a Full Professor of Analytical Chemistry and Chemometrics. He belongs to the National Council of Scientific and Technical Research (CONICET) of Argentina. His research interests include development of analytical methods based on fluorescence spectroscopy and separations coupled to chemometric modelling.

Reyes Babiano received his PhD degree in Chemistry from the University of Extremadura, where he is currently Associate Professor of Organic Chemistry. He has published over 100 papers in peer-reviewed journals. His research interests focus on different domains of basic and applied chemistry, such as development of asymmetric transformations with chiral auxiliaries, practical and theoretical aspects of chirality, molecular recognition, gelation phenomena, chemical sensors, and improved materials via *surface modification.*

Table 1 Spectroscopic and analytical parameters of the rhodamine 6G derivative chemodosimeters/chemosensors proposed for Hg^{2+} detection



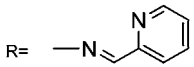
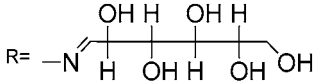
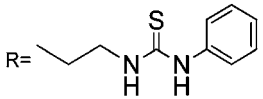
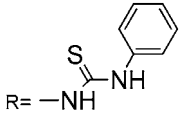
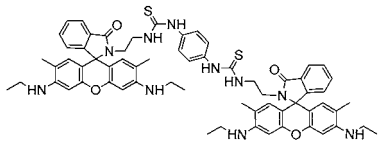
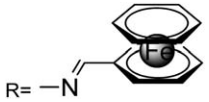
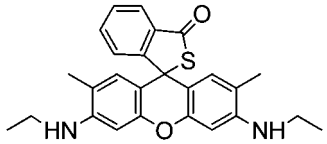
Sensor compound	Structure	∅	[R] (μM)	Reaction medium	λ_{exc} (nm)	λ_{em} (nm)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Reversibility	Analytical applications	Ref.
RG1		0.42	5	DMF–H_2O (50 : 50 v/v), pH 5–10	500	560	2–20	2	—	2 : 1	—	—	36
RG2		0.48	0.1	H_2O, pH 5–10	500	550	2–10	1	—	1 : 1	Yes, by adding Na_2S	Living cells and natural waters (seawater, drinking water)	37
RG3		—	10	MetOH–H_2O (90 : 10 v/v), pH 5–10 (pH 7)	520	555	(2–100) × 10^3	—	—	1 : 1	Irreversible	—	38
RG4		0.52	0.1	MetOH–H_2O (80 : 20 v/v), pH 4–14 (pH 7.4)	515	557	1–12	0.7	—	1 : 1	Irreversible	Living cells and zebra fish organs, water and fish samples	39–46
RG5		—	0.1	DMSO–MetOH (10 : 90 v/v), pH 5–10	520	550	1–9	1	Slight interference from Ag^+, Zn^{2+}, Cu^{2+}	1 : 2	Irreversible	Living cells	47
RG6		0.38	0.1	H_2O, pH 5.5–12 (pH 7)	500	550	1–5	1	—	1 : 1	Yes, by adding Na_2S	Natural waters	48
RG7		—	10		515	545	0.2–2	—	—	2 : 1	Yes, by adding KI	*In vivo* analysis using *C. elegans*, adapted for portable LED based fluorimeters	49 and 50

Table 1 (*Contd.*)

Sensor compound	Structure	[R] (μM) ∅	Reaction medium	λ_{exc} (nm)	λ_{em} (nm)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Reversibility	Analytical applications	Ref.
RG8		— 5	DMF–H_2O, (20 : 80 v/v), pH 6–7.4 (pH 7.2)	500	566	10–800	8	—	1 : 1	Partial reversibility	Living cells	51
RG9		— 5	EtOH–H_2O, (1 : 4 v/v), pH 7	500	556	(0.1–2) × 10^3	—	—	1 : 1	—	—	52

dosimeter molecule and the target analyte leading to an observable signal (some physical change), in which an accumulative effect is directly related to the analyte concentration.

On the other hand, chemosensors are molecules that interact with the analyte to yield measurable signals with a real-time response (usually less than a few seconds). Chemosensors, contrarily to chemodosimeters, work in a general operating principle which is based on coordination events. Consequently, the reaction of a chemosensor with the target analyte and the accompanying signal changes are reversible, which is the main difference between chemosensors and chemodosimeters.[31] It is important to remark that both kinds of sensors should ideally be selective for a particular guest and not only report the presence of the analyte, but should also allow monitoring its concentration. This is important medically (for monitoring indicators of physical functions) and environmentally (monitoring pollutant levels). Frequently, the constitution of sensors with adequate properties is only possible through the design of suitable abiotic receptors.

Fluorogenic chemodosimeters and chemosensors can report the presence of an analyte *via* changes in the measurable photophysical property of the system and, as stated above, both quenching and enhancement of fluorescence can be used to detect the presence of an analyte, although for practical reasons enhancement is preferable in sensors as the decreased emissions have low signal outputs upon interaction with the analyte of interest.

As was previously mentioned, chemodosimeters/chemosensors are potentially useful in chemical as well as biological systems. Especially, the use of sensor molecules for Hg^{2+} that present instantaneous measurable optical response is highly attractive.

Interestingly, sensitivity of fluorescence techniques is important in this context. While absorbance measurements for colorimetric sensors can reliably determine concentrations only as low as several tenths of a micromole, fluorescence techniques can accurately measure concentrations one million times smaller. Due to these advantages fluorescent sensors are especially attractive as they give a meaningful physical output which is easy to measure even at low concentrations. Thus, fluorescent chemosensor design is an active field of supramolecular chemistry, not only because of potential practical benefits in cell physiology and analytical and environmental chemistry, but also as a proving ground for manipulation and/or engineering of various photophysical processes toward an ultimate goal of selective and sensitive signaling of targeted molecular or ionic species.[32,33]

3 Rhodamine-based chemodosimeters and chemosensors for Hg^{2+}

Rhodamine spirolactam based molecular probes have attracted a lot of attention since the pioneering work of Czarnik *et al.*[30] Since then, a number of rhodamine derivatives have been synthesized as chemodosimeters and/or chemosensors for different metal ions including mercury. Recent reviews deal

with the detection of Hg^{2+} and other ions with rhodamine based sensors.[34,35]

A particularly attractive approach is the use of fluorimetric chemodosimeters for the determination of Hg^{2+}, through a specific chemical reaction between dosimeter molecules and the target species. This involves the use of highly selective reactions that can be reversible or irreversible, induced by the presence of mercury ions, in which a fluorescence enhancement effect is directly related to analyte concentration. In Tables 1 and 2, the reported molecular probes, the spectroscopic and analytical parameters and practical applications of the proposed rhodamine 6G and B derivatives for Hg^{2+} determination are summarized.

3.1 Rhodamine 6G derivatives

Most of the rhodamine-derived chemical sensors follow the approach of "spirolactam ring opening" for the detection of mercury ions, as the rhodamine derivatives with a spirolactam structure are non-fluorescent, whereas ring-opening of the spirolactam will give rise to a strong fluorescence emission.

Usually, rhodamine system based sensors follow the mechanism shown in Scheme 1. This system is formed by the rhodamine fluorophore linked to a mercury receptor (dashed line rectangle) with different structures in each sensor. In the absence of the analyte, the sensor fluorophore has the spirolactamic structure, with a carbon atom with sp^3 hybridization that prevents planarity and electronic delocalization between aromatic rings. After Hg^{2+} complexation in the receptor, a strong structural change is produced, which implies C–N spiranic bond rupture and formation of dideoxidiaminofluorone rings of rhodamines.

Specificity and binding to the rhodamine derivatives depend on the type and spatial distribution of the donors attached and are frequently influenced by the nature of the solvents used.

Among the rhodamine 6G derivatives, Wu *et al.* reported a highly sensitive fluorescence probe (sensor RG1) containing a carbohydrazone binding unit, selective for Hg^{2+} in mixed dimethylformamide (DMF) aqueous media,[36] detecting ng mL^{-1} of Hg^{2+}. The proposed mechanism of fluorescence enhancement of RG1 upon the addition of Hg^{2+} involves the formation of a ring-opened amide form from the initial spirolactam form (Scheme 2). No significant spectral changes of the sensor occur in the presence of a number of other ions, due to several combined influences cooperating to achieve the unique selectivity for Hg^{2+} ions, such as the suitable coordination geometry conformation of the bischelating Schiff-base receptor, the larger radius of the Hg^{2+} ion, and the nitrogen-affinity character of the Hg^{2+} ion. A 2 : 1 stoichiometry R : Hg^{2+} was proposed and the complexation mechanism is shown in Scheme 2. The proposed probe was not applied in real samples.

By combination of a water soluble sugar group and a rhodamine group in a molecule, a bright water compatible and specific sensor for Hg^{2+} (sensor RG2) in natural waters and living cells was achieved with a 1 ng mL^{-1} limit of detection, useful for monitoring Hg^{2+} within biological samples.[37] Changes in the fluorescence band suggest that as a result from RG2–Hg^{2+} binding, a xanthene moiety is formed after the spiro ring is opened. The chemosensor was applied to Hg^{2+} determination in spiked natural water samples, seawater from Yellow Sea and freshwater from West Hill reservoir (Dalian, area of china), with satisfactory results. Also, the practical ability of RG2 as a Hg^{2+} probe was demonstrated in the fluorescence imaging of HeLa living cells.

Sensor RG3 was reported for Hg^{2+} chemosensing inducing a new 555 nm peak through the formation of a stable cyclic product by an irreversible desulfurization reaction,[38] requiring more than 10 minutes to complete the reaction. No practical application was described. From the molecular structure, it is concluded that the addition of the Hg^{2+} ion induced the N atom of the spirolactam to attack the C atom of the thiourea, and thus a ring opening of the spirolactam rhodamine took place, followed by a removal of HgS and the formation of intramolecular guanylation (Scheme 3).

Yang *et al.* first reported a fluorescent and colorimetric rhodamine-6G derivative (sensor RG4) that uses promoted desulfurization and cyclization reaction giving rise to 26-fold fluorescence enhancement and a high quantum yield of the reaction product.[39] The RG4–Hg^{2+} interaction facilitates the spyrocycle ring opening and allows thiosemicarbazide to oxadiazole transformation (Scheme 4). The system was successfully applied to monitor exogenous Hg^{2+} uptake in living cells and vertebrate organisms in real time[40,41] and also applied for methylmercury detection.[42] Later on, it was applied to the determination of Hg^{2+} in waters and fish samples, the fluorescence intensity being proportional to the amount of Hg^{2+} at ng mL^{-1} levels, capable of distinguishing between safe and toxic levels of inorganic mercury. The procedure was implemented in a portable instrument composed of a 515 nm light-emitting diode (LED) excitation source, two filter optics, and a charge-coupled device (CCD) camera as detector, connected to a portable computer for data acquisition and analysis, intended for *in situ* determination of mercury, and offering a viable alternative to a conventional spectrofluorimeter.[43]

This chemodosimeter has been also immobilized in nylon membranes and applied to the determination of Hg^{2+} in mineral, underground and river water samples, enhancing the sensitivity of the reaction with a LOD of 0.4 ng mL^{-1}.[44,45] Also, the probe has been immobilized in a polymeric membrane by a reverse atom transfer radical polymerization (ATRP) technique, and it is constituted by methylmethacrylate (MMA) and hydroxyethylmethacrylate (HEMA) units, as a preliminary step for the development of a new Hg^{2+}-sensing film.[46]

Xi *et al.* reported sensor RG5 with a remarkably high selectivity and a LOD of 2 ng mL^{-1} in dimethylsulfoxide (DMSO) : methanol solutions[47] that was applied to HeLa living cells to map its subcellular distribution, localizing the fluorescence signals in the perinuclear area of cytosol. The authors used a multichannel molecular system that incorporated two rhodamine fluorophores into a diisothiocyanate molecule to form a dual-rhodamine urea, instead of a common monorhodamine chemodosimeter system. Spectral changes in the emission band after Hg^{2+} addition can be attributed to delocalization in the xanthene moiety *via* spirocyclic opening.

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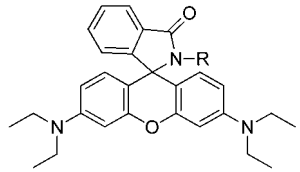
Table 2 Spectroscopic and analytical parameters of the rhodamine B derivative chemodosimeters/chemosensors proposed for Hg^{2+} detection



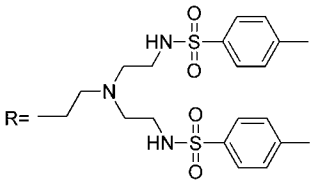
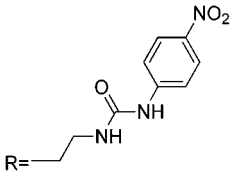
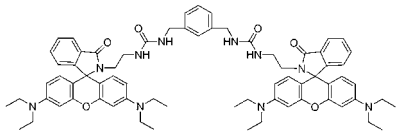
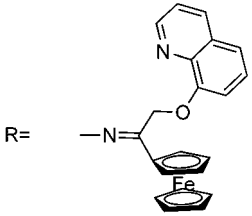
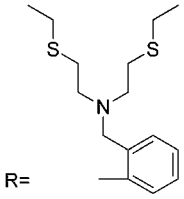
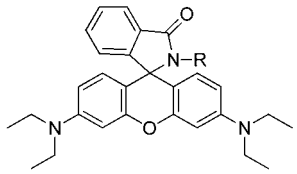
Sensor compound	Structure	∅	[R] (μM)	Reaction medium	λ_{exc} (nm)	λ_{em} (nm)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Reversibility	Analytical applications	Ref.
RB1		—	1	CH_3CN : H_2O, (90 : 10 v/v), pH 3–6	520	575	(0.2–2) × 10^3		—	1 : 1	—	—	53
RB2		—	6	CH_3CN : H_2O (95 : 5 v/v)	554	575	(0.2–12) × 10^3	—	Cd^{2+},Zn^{2+}, Pb^{2+}	1 : 1	Reversible	—	54
RB3		—	6	CH_3CN : H_2O (95 : 5 v/v)	554	575	—	—	Zn^{2+}	1 : 1	Reversible	—	54
RB4		0.15	20	EtOH–H_2O (50 : 50 v/v), pH 7.2	520	580	—	—	—	1 : 1	Yes, by adding I^-	Living cells	55
RB5		—	5	MeCN/HEPES, (15/85), pH 6–12 (pH 6.98)	530	597	—	—	—	1 : 1	Yes, by adding EDTA	—	56

Table 2 (*Contd.*)



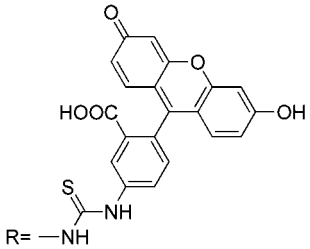
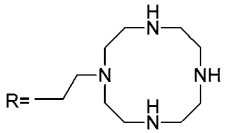
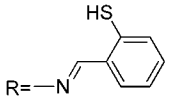
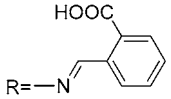
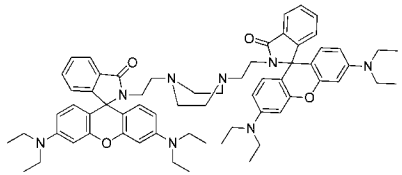
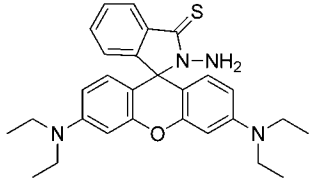
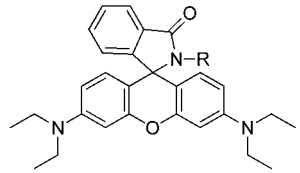
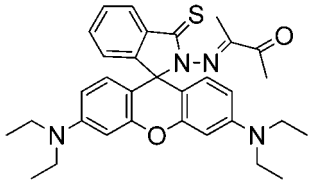
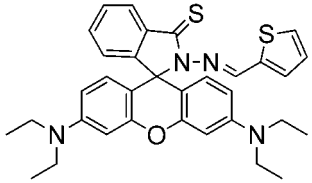
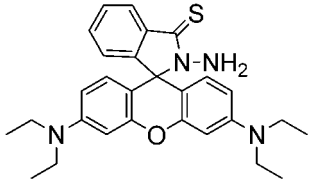
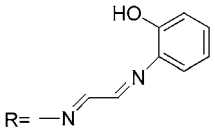
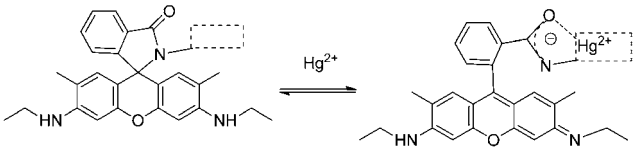
Sensor compound	Structure	∅	[R] (μM)	Reaction medium	λ_{exc} (nm)	λ_{em} (nm)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Reversibility	Analytical applications	Ref.
RB6		—	10	H_2O, pH 5.5–12 (pH 7)	490	FRET ratio: 591/520	0–200	10	Cr^{3+}	1 : 1	Irreversible	—	57
RB7		0.19	5	CH_3CN	530	580	(3–13) × 10^3	—	—	1 : 2	Yes, by adding triethylene-tetramine	—	58
RB8		—	10	CH_3CH : H_2O (1 : 99 v/v)	510	580	0.2–200	0.2	—	2 : 1	Yes, by adding KI	*In vivo C. elegans*	59
RB9		—	10	CH_3CH : H_2O (1 : 99 v/v)	510	578	0.2–200	0.8	—	1 : 1	Yes, by adding KI	*In vivo C. elegans*	59
RB10		0.51	10	EtOH : H_2O (50 : 50 v/v)	520	575	(0.4–4) × 10^3	—	—	1 : 2	—	—	60
RB11		—	10	1,4-Dioxane : H_2O, (1 : 99), pH 3.40	530	582	80–600	—	—	2 : 1	Yes, by adding KI	—	61

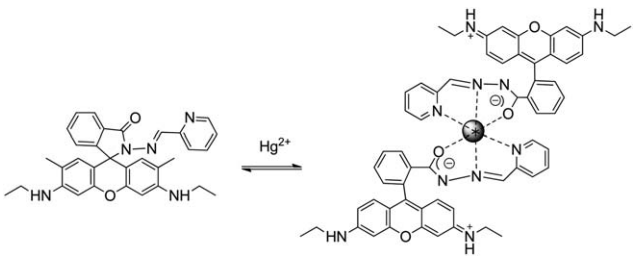
Table 2 (*Contd.*)



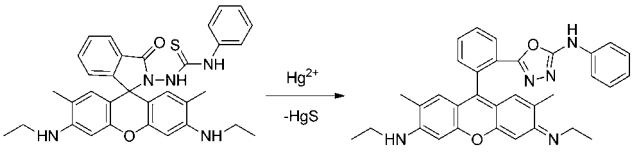
Sensor compound	Structure	∅	[R] (μM)	Reaction medium	λ_{exc} (nm)	λ_{em} (nm)	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Reversibility	Analytical applications	Ref.
RB12		—	5	H_2O, pH 4–10 (pH 7.4)	510	591	—	—	Cu^{2+}	1 : 1	Yes, by adding Na_2S	Living cells	62
RB13		—	1	EtOH : H_2O (50 : 50 v/v), pH 5.58–9.21 (pH 7.0)	515	593	1–6	0.4	Slight interference from Ag^+ and Cu^{2+}	1 : 1	Yes, by adding Na_2S	Living cells	63
RB14		—	5	1-4Dioxane : H_2O (0.5 : 99.5 v/v), pH 3–11	530	585	(1–10) × 10^2	4	Slight interference from Ag^+	2 : 1 and 1 : 1	Depending on the Hg^{2+} concentration by adding KI	—	64
		—	10	CH_3CN–H_2O (1 : 99 v/v), pH 4	530	585	(4–40) × 10^3	—	—	2 : 1	Yes, by adding KI	—	65
RB15		0.75	5	EtOH : H_2O (50 : 50 v/v), pH 7	510	580	1–5	0.9	—	1 : 1	Irreversible	Natural water samples and living cells	66



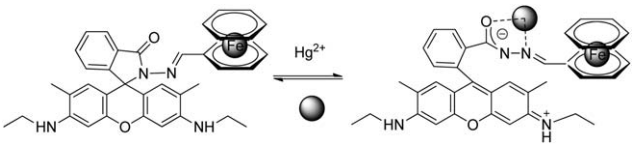
Scheme 1 Spiranic system opening induced by Hg^{2+} complexation.



Scheme 2 Proposed binding mode of RG1 with Hg^{2+}.



Scheme 4 Proposed mechanism of Hg^{2+} induced ring opening and cyclization of RG4.



Scheme 5 Proposed binding mode of RG6 with Hg^{2+}.

A new chemosensor combining a ferrocene unit and a rhodamine 6G derivative block *via* the linkage of carbohydrazone binding units was prepared for the detection of Hg^{2+} in natural water with a LOD of 1 ng mL^{-1}. The sensor–Hg^{2+} complex was isolated and it was demonstrated that the Hg^{2+} binding is reversible[48] (sensor RG6), and needs previous spirocyclic opening (Scheme 5). The reported dye meets the criteria of appropriate selectivity over the competing metal ion contaminants and of optical sensitivity in an aqueous solution, and was applied to Hg^{2+} determination in spiked seawater from the Yellow Sea and freshwater from the West Hill Reservoir (Dalian, area of China).

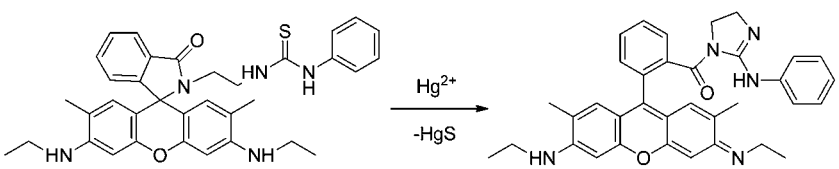
Chen *et al.* reported a rhodamine 6G thiolactone derivative sensor RG7 detecting Hg^{2+} in the nanomolar range in neutral aqueous solutions which was applied for *in vivo* imaging of Hg^{2+} using the bacteriovorous nematode *C. elegans*, which has been considered as an ideal organism for testing the toxicity of aquatic media such as municipal and industrial wastewater.[49] Later on, the chemosensor was adapted to a light emitting diode (LED) exciting, fiber-optic and charged coupled device (CCD) based portable spectrofluorimeter, for analysis of environmental water samples.[50] The large fluorescence enhancement is due to the induced spirothiolactone ring opening *via* Hg^{2+} complexation (Scheme 6).

A new fluorescence turn-on probe (sensor RG8) for Hg^{2+} imaging in living cells was designed based on the interactions of Hg^{2+} with both thiol and alkyne moieties in a rhodamine 6G scaffold. The selection of thiol and alkyne moieties was based on the thiophilic and π-philic nature of Hg^{2+}.[51] The probe exhibits large fluorescence enhancement (140-fold), high selectivity, low detection limit and fast response, as the fluorescence enhancement reached the maximum within 3 minutes, and was satisfactorily applied for Hg^{2+} imaging in human Tca-8113 living cells. The proposed mechanism of the Hg^{2+}-induced turn-on response is shown in Scheme 7, as concluded by mass-spectrometry and NMR analysis, thiozolcarbaldehyde being the minor product. The mechanism is consistent with the fact that when IK is added to the probe + Hg^{2+}, only partial fluorescence quenching was noted, indicating that the sensing process is not completely reversible.

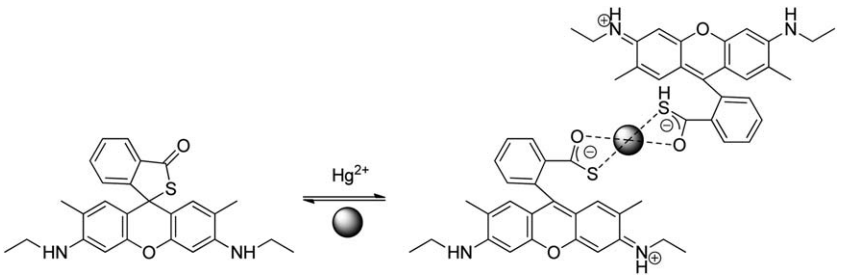
A new rhodamine 6G-derived Schiff base (sensor RG9) was reported as a chemosensor with good selectivity towards Hg^{2+}, and a wide pH span (6–10), giving a good linear relationship in the concentration range from 0.5 to 10 μM, although the sensitivity was poor and it was not applied to real samples.[52] From the molecular results and spectral changes observed, it was concluded that the addition of Hg^{2+} ions induced its complexation with carbonyl groups in spirolactam, N atoms in Schiff base, and hydroxyl in salicylaldehyde, as depicted in Scheme 8.

3.2 Rhodamine B derivatives

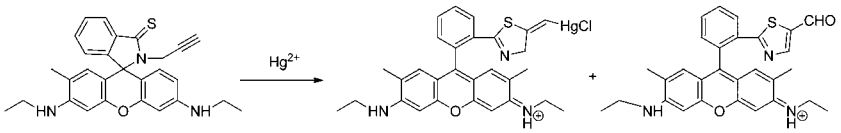
Lee *et al.* reported sensor RB1 as a *N*-tripodal structural compound consisting of a rhodamine B moiety and two branched tosyl group derivatives that is sensitive to Hg^{2+} in a CH_3CN solution.[53] The structure of the tripodaltris(2-aminoethyl)amine moiety (tren) has a good chelating ability for Hg^{2+} ions. The spirolactam-structured rhodamine moiety is directly



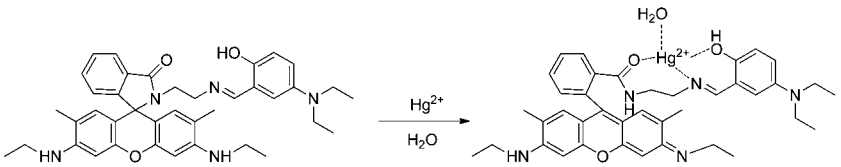
Scheme 3 Proposed mechanism of Hg^{2+}-induced ring opening and cyclization of RG3.



Scheme 6 Proposed binding mode of RG7 with Hg^{2+}.

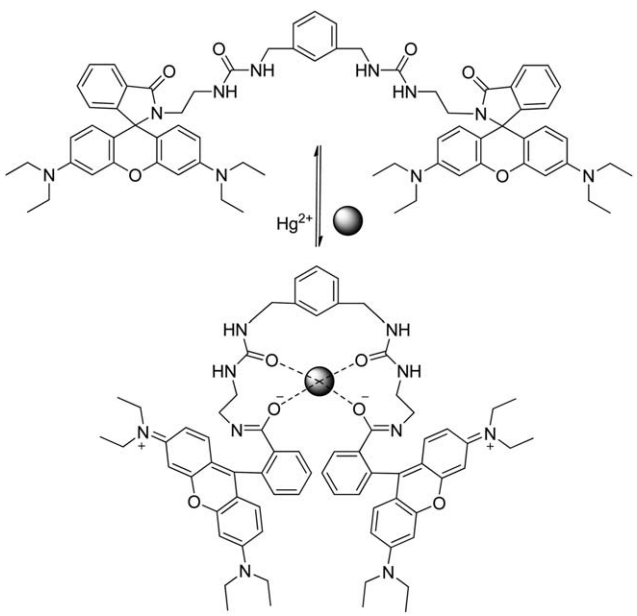


Scheme 7 Proposed binding mode of RG8 with Hg^{2+}.



Scheme 8 Proposed binding mode of RG9 with Hg^{2+}.

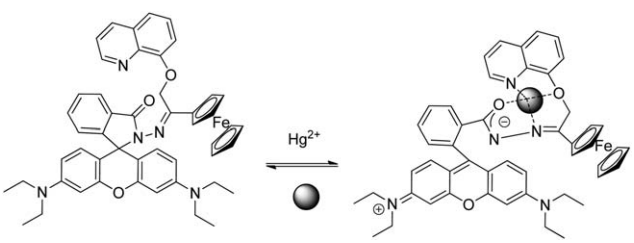
linked to tren, and when the binding event takes place, it also leads to a ring-opening of spirolactam along with an obvious OFF–ON optical signal, and the strong electron-acceptor-two tosyl groups further produce a strong binding ability with Hg^{2+}. Application to real samples was not reported.



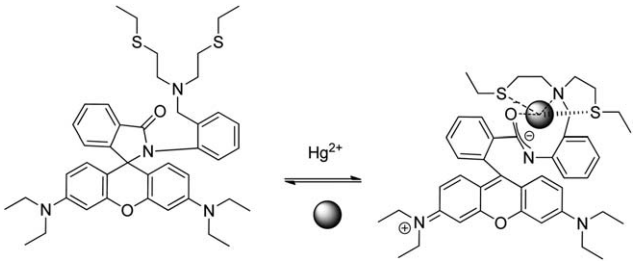
Scheme 9 Proposed binding mode of RB3 with Hg^{2+}.

Two different rhodamine derivatives bearing the urea groups (sensors RB2–RB3) were reported by Soh *et al.*[54] The sensor RB2 also exhibits reaction with Cd^{2+}, Zn^{2+} and Pb^{2+}, whereas sensor RB3 exhibits reaction with Zn^{2+}. The sensitivity is poor in both cases and the dimmeric system displayed a higher selective fluorescent enhancement upon the addition of Hg^{2+}. Complexation with metal ions forces ring opening to conjugated xantene moiety, and the two carbonyl oxygens provide a nice binding pocket for Hg^{2+} as shown in Scheme 9. Application of the probe to real samples was not reported.

A new multisignaling optical–electrochemical sensor based on rhodamine B with a ferrocene subunit (sensor RB4) has been synthesized and has been shown to display extreme selectivity for Hg^{2+} over other metal ions. Multisignaling changes are observed through UV absorption, fluorescence emission, and electrochemical measurements, and applied for monitoring Hg^{2+} in living cells.[55] The reaction is reversible (Scheme 10), which implies that RB4 acts as a chemosensor and not as a



Scheme 10 Proposed binding mode of RB4 with Hg^{2+}.



Scheme 11 Proposed binding mode of RB5 with Hg^{2+}.

chemodosimeter for Hg^{2+}. Laser confocal fluorescence microscopy was used for Hg^{2+} monitoring in Caor-3 ovarian carcinoma cells, showing that the fluorescence signals are localized in the perinuclear area of cytosol, indicating a subcellular distribution of Hg^{2+}.

Huang *et al.* reported a rhodamine fluorophore (sensor RB5) by incorporation of ionophore NS_2, which was established to be reversible by EDTA addition,[56] as a result of the regeneration of the spirocyclic moiety (Scheme 11). The receptor contained the NS_2 fragment, which is a well-known specific and reversible binding receptor of Hg^{2+} due to the thiophilic nature of Hg^{2+}. Compared with other rhodamine-based chemosensors, RB5 reduced the amount of organic co-solvent in detecting media incorporating sensitivity, although application of the probe to real samples was not reported.

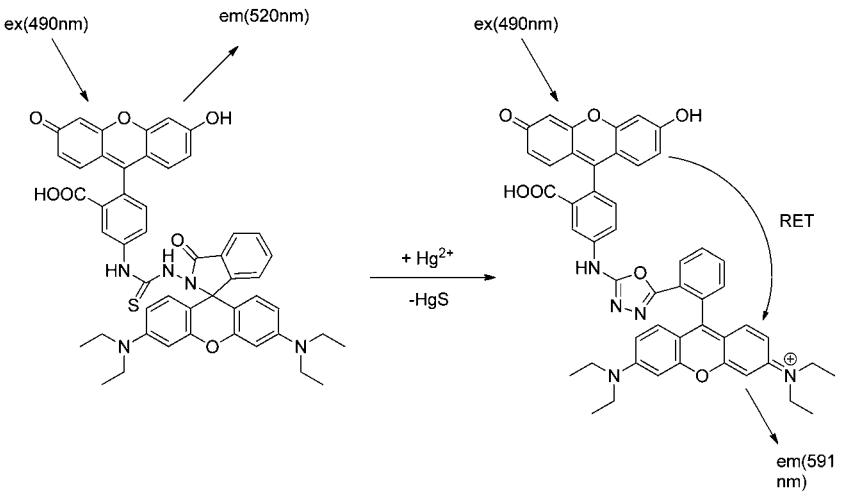
Based on an intramolecular fluorescence resonance energy transfer (RET) mechanism, Shang *et al.* reported a new fluorescence probe (sensor RB6) for Hg^{2+}. The fluorescence probe included a fluorescein fluorophore linked to a rhodamine B hydrazide by a thiourea spacer. The color of this probe changes from yellow to magenta when reacted with Hg^{2+}, which allows mercury detection by ratiometric fluorometry by measuring the fluorescence at a 591 nm/520 nm ratio.[57] HgS is generated in the reaction due to oxadiazole cyclization after spirolactam ring opening. No application to real samples was reported. RET is an interaction between a fluorophore at the electronic excited state (energy donor) and a fluorophore at the ground state (energy acceptor), which leads to the transfer of excitation energy from the donor to the acceptor. The free fluorescence probe showed a maximum absorption wavelength at 490 nm, which exhibited a slightly yellow color dominated by the fluorescein chromophore, and no intramolecular RET phenomenon was observed. Therefore, only green fluorescence (520 nm) of fluorescein itself was observed when the probe was excited at 490 nm. The reaction of Hg^{2+} at the thiosemicarbazide group forces the molecule to form a 1,3,4-oxadiazole group as a new spacer and leads to the release of a fluorescent rhodamine B moiety, which triggers an intramolecular RET with a high selectivity, as shown in Scheme 12.

A rhodamine–cyclen conjugate that behaves as a highly sensitive and selective chemosensor (sensor RB7) for Hg^{2+} has been reported by Shiraishi *et al.*[58] The high emission sensitivity is due to the formation of a 1 : 2 complex leading to spirocycle opening of the sensor along with an appearance of strong orange fluorescence and a clear color change from colorless to pink. The fluorescence enhancement is 1700 and no application to real samples was reported.

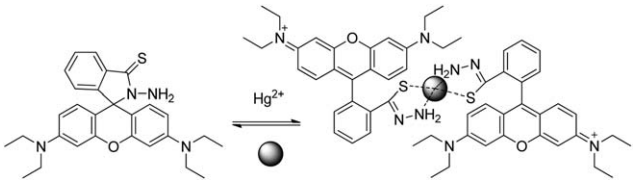
New rhodamine hydrazine derivatives bearing thiol and carboxylic acid groups have been reported (sensors RB8–RB9).[59] They enable the visualization of Hg^{2+} accumulated in the nematode *C. elegans*, commonly used to test and evaluate the toxicity level of heavy atoms, previously exposed to nanomolar concentrations of Hg^{2+}.

A bis-rhodaminepiperazine conjugate has been synthesized that in the presence of Hg^{2+} specifically changes from colorless to pink and exhibits high fluorescence upon excitation at 520 nm (ref. 60) (sensor RB10). The presence of other relevant metal ions in the system does not affect the fluorescence output to any significant extent and the compound can be used as a chromogenic and fluorogenic sensor for Hg^{2+} ions in an aqueous ethanol medium, although no application to real samples was reported.

Zheng *et al.*[61] reported a reversible Hg^{2+} ion sensor RB11 based on rhodamine B thiohydrazide, containing an S atom and



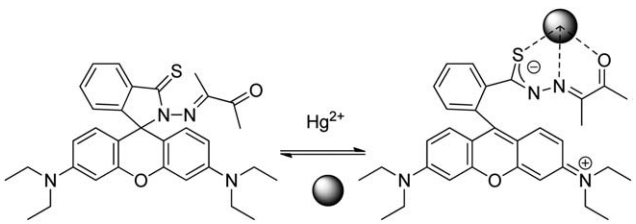
Scheme 12 Proposed mechanism of Hg^{2+}-induced ring opening and cyclization of the RB6 and RET-based detection mechanism of Hg^{2+}.



Scheme 13 Proposed binding mode of RB11 with Hg^{2+}.

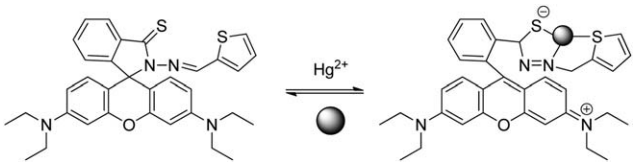
a $-NH_2$ group attached to the N-bearing spiro ring. The sensor RB11 is a highly selective sensor. It shows 7-fold fluorescent enhancement only in the presence of Hg^{2+}, due to delocalization in the xanthene moiety generated after cycle rupture (Scheme 13). No application to real samples is reported to date.

Later on, Wang *et al.*[62] reported a fluorescent sensor based on thiooxorhodamine B (sensor RB12) to detect Hg^{2+} in an aqueous buffer solution. It demonstrated high selectivity for sensing Hg^{2+} with about 383-fold enhancement of emission intensity and micromolar sensitivity. The sensor is cell permeable and can visualize the changes in intracellular mercury ions in living cells using fluorescence microscopy, as probed by confocal fluorescence images of intracellular Hg^{2+} in A549 cells. A reversible mechanism based on complexation with mercuric ions is proposed (Scheme 14).



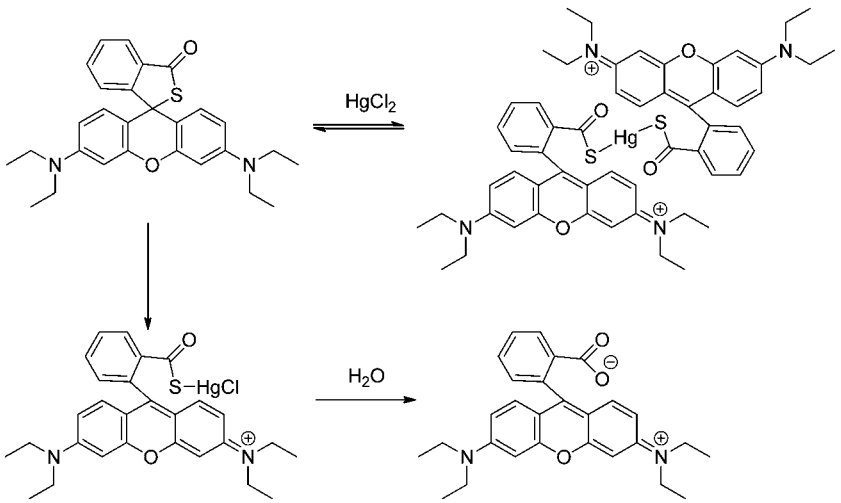
Scheme 14 Proposed binding mode of RB12 with Hg^{2+}.

A rhodamine B based sensor (sensor RB13) was synthesized by combination of the thiospirolactam chromophore and the thiophene ring block with high affinity to Hg^{2+} in an aqueous solution. The introduction of the thiophene ring increases the affinity to Hg^{2+} ions in competitive media, changes the spatial effects within one molecule, allows real-time detection, as it quickly induces the fluorescence and color response, and improves the sensitivity of Hg^{2+} ions.[63] Modification in the absorption spectra after the addition of Hg^{2+} suggests the formation of a ring-opened form. The proposed reversible mechanism, based on a 1 : 1 stoichiometry, is shown in Scheme 15.

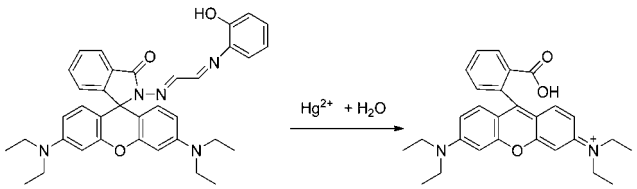


Scheme 15 Proposed binding mode of RB13 with Hg^{2+}.

A rhodamine B thiolactone (sensor RB14) was also reported as a highly selective and sensitive sensor for Hg^{2+} and could be used for naked-eye detection in an aqueous solution.[64,65] The change in colour can be explained to be a result of delocalization of the xanthene moiety of rhodamine due to Hg^{2+} binding. The chemosensor, reported by two different groups, was readily prepared and found to be stable in both alkaline and acidic solutions. The spectral response towards Hg^{2+} was established to be reversible by adding KI in a water : CH_3CN (99 : 1% v/v) medium.[65] However, in 1,4-dioxane : water (0.5 : 99.5% v/v),[64] the introduction of KI into the system can reverse the reaction only in the presence of less than 0.5 equivalents of Hg^{2+}. The reaction scheme in this system proceeds through the route depicted in Scheme 16, in which the high thiophilicity of Hg^{2+} leads to the formation of two kinds of complexes of stoichiometries 2 : 1 R : Hg^{2+} and 1 : 1 R : Hg^{2+}. The 2 : 1 complex is relatively stable in solution, but the 1 : 1 complex can be further degraded to rhodamine B. No application to real samples was reported.



Scheme 16 Proposed binding mode of RB14 with Hg^{2+}.



Scheme 17 Proposed mechanism of Hg^{2+}-induced ring opening and hydrolysis of RB15.

On the basis of the mechanism of Hg^{2+} promoted hydrolysis (Scheme 17), a new chemodosimeter has been reported (sensor RB15), and applied to Hg^{2+} determination in living cells with satisfactory results. The probe has a limit of detection of 0.9 ng mL^{-1}, below the toxic levels of Hg^{2+} in drinking water in a 1 : 1 ethanol–water reaction medium,[66] and was also applied to natural water samples from three different sources: seawater from Yellow Sea (Dalian, area of China), pool water and tap water.

4 BODIPY-based chemosensors and chemodosimeters for Hg^{2+}

4.1 Photoinduced electron transfer (PET)

Photoinduced electron transfer (PET) sensors are an important family of chemosensors. Small molecule fluorescence turn-on sensors have been developed and studied on the basis of the PET mechanism. The general mechanism of fluorescent indicator devices based on PET combines an analyte recognition site (also called chelating group, coordination site, receptor, host or ligand) with a fluorophore, *i.e.* a fluorescent molecule which translates the binding between the analyte and the recognition site into a fluorescence output signal.[67]

Changes in absorption or emission spectra are the most common ones among the possible signal modalities. In this regard, the PET process is particularly useful, as the signal, depending on the special circumstances, is either an "on–off" or "off–on" type,[68,69] resulting in a well-defined response. An interesting fact is that PET produces very sharp changes in the signal intensity, while keeping the emission wavelength unchanged.

For PET sensors the receptor and fluorophore are usually separated by a (short) spacer electronically connecting the π-electron systems of receptor and fluorophore. In such systems, the receptors usually contain a relatively high-energy nonbonding electron pair. In the unbound state, after excitation, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), which enables a fast intramolecular electron transfer from the HOMO of the receptor (donor) to the LUMO of the excited fluorophore. Such a process provides a mechanism for nonradiative deactivation of the excited state, causing a quenching effect of the fluorescence of the system. When the receptor is bound, this electron pair is coordinated to Lewis acid cations in solution, the HOMO of the receptor is lowered becoming lower than that of the fluorophore, the receptor redox potential is perturbed and slows down the PET process, reviving fluorescence emission; this logic indication can also be reversed.[70]

Fig. 1 shows a three module format in which a reductive PET is produced by permitting electron transfer from the receptor to the fluorophore, if the process is thermodynamically and kinetically feasible.[67] The electron transfer rate in many favorable cases is much faster than the luminescence rate when PET is thermodynamically allowed. Luminescence and electron transfer are the two main competitors, which deactivate the photoexcited state of these designed systems. Binding of the target species (typically a metal ion) to the receptor can drastically alter PET thermodynamics to an endergonic situation. At the simplest level, this situation is caused by electrostatic interactions between the receptor–target pair. Luminescence is now the winner of the competition and thus can be switched between "on" and "off" states by introduction and removal,

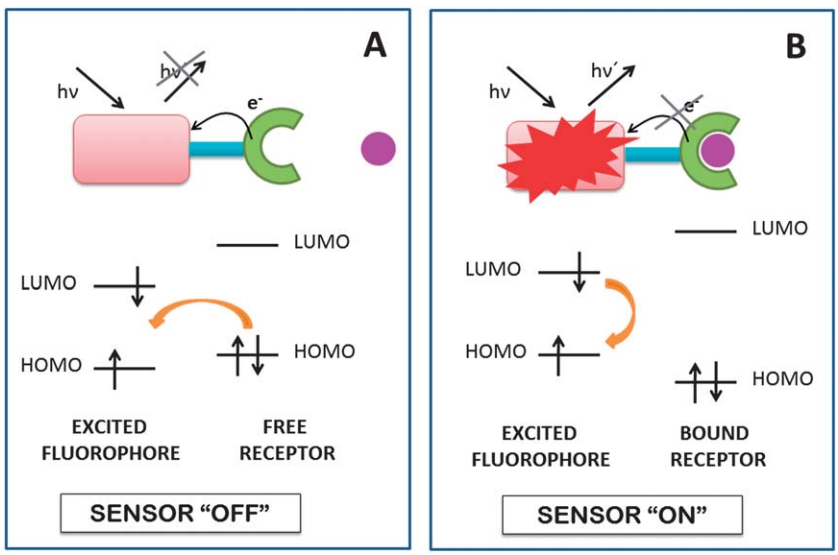


Fig. 1 Reductive PET for fluorescent indicators. (A) The three module format allows electron transfer from the chelator to the fluorophore, switching off the sensor. (B) When the receptor is bound, the receptor redox potential is perturbed and slows down the PET process, switching on the sensor.

respectively, of the target species, which provides us with the responsive mechanism we needed (Fig. 1). Mechanistically, the fluorophore–receptor pair (Fig. 1A) is selected to allow rapid PET between them on the basis of simple electrochemical criteria. The rapidity is usually assured by sufficiently favorable PET thermodynamics and by using sufficiently short spacers. However, the designer must also allow for the reversal of PET thermodynamics in the guest-bound situation (Fig. 1B). Guest-induced conformational changes in the receptor can be valuable adjunct to purely electrostatic effects for the facilitation of sensing. The clearest result is a fluorescence switching "off–on" effect caused by the guest.[67]

4.2 BODIPY mechanism for Hg^{2+} chemosensing and derivatives

Since their discovery in 1968 by Treibs *et al.*,[71] BODIPY dyes (4,4-difluoro-4-bora-3a, 4a-diaza-s-indacene, difluoroborondipyrromethene) have become very popular in the fluorescent

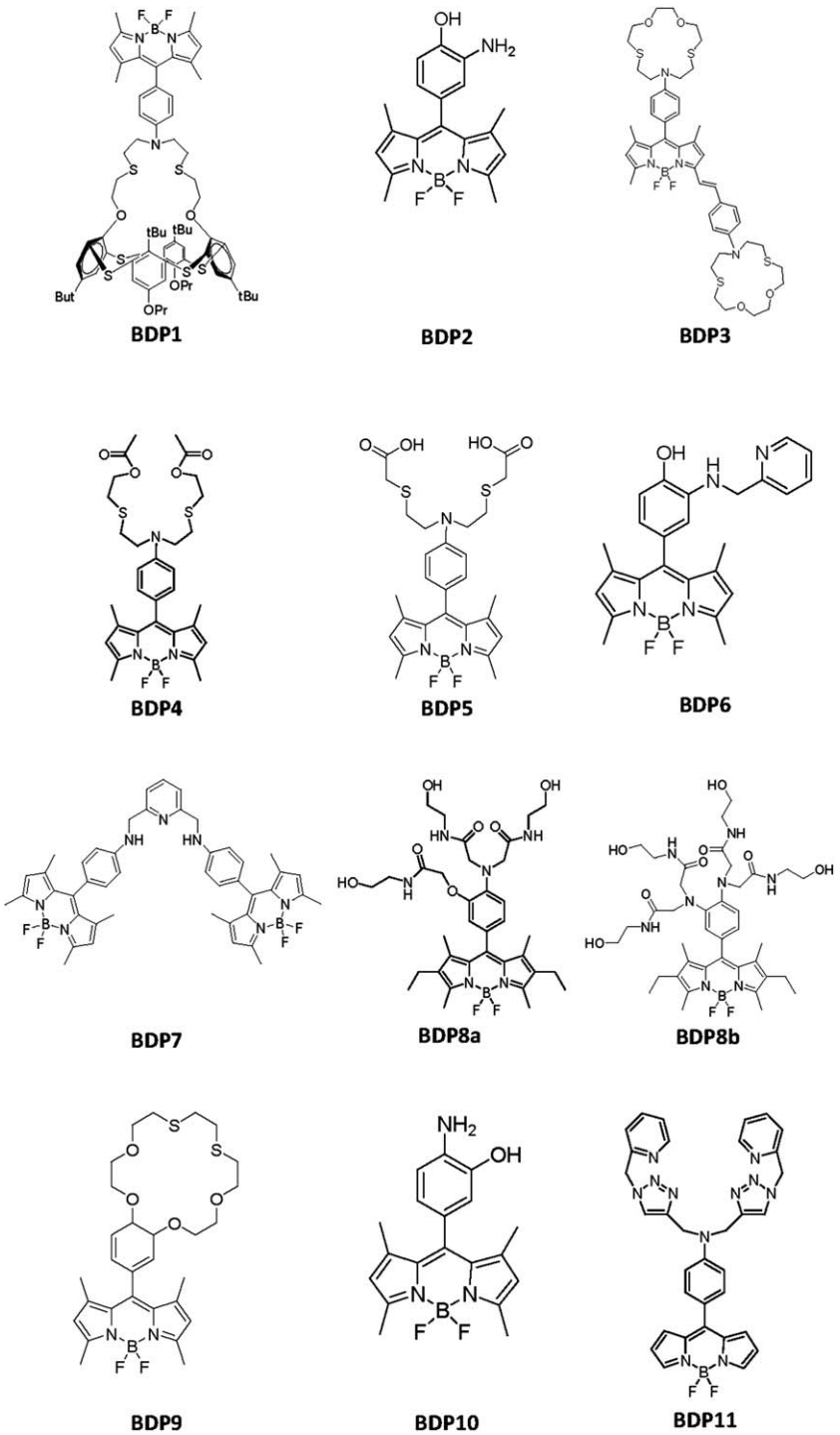
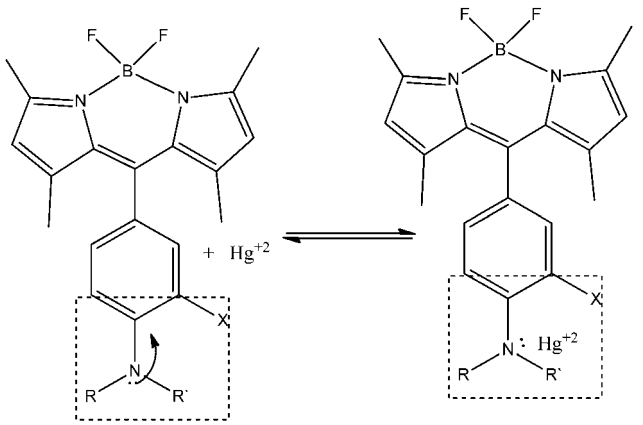


Fig. 2 Chemical structures of the BODIPY derivatives included in Table 3.



Scheme 18 Mechanism of BODIPY PET sensors for Hg^{2+} determination.

chemosensor field due to their notable properties, starting from the relatively moderate redox potential of the BODIPY core (see Fig. 2), which is a requisite in the construction of fluorescent probes based on electron transfer processes.[70]

The excellent luminescent properties of the BODIPY dyes included their photochemical stability, the relatively high molar absorption coefficients and fluorescence quantum yields, insignificant triplet-state formation, excitation and emission wavelengths in the visible spectral region with high intensity narrow emission peaks, good solubility and resistance toward self-aggregation in solution and fluorescence lifetimes in the nanosecond range.[72–74]

Chemosensors based on the BODIPY system follow the mechanism showed on Scheme 18. The fluorophore is difluoroborondipyrromethene. It is connected to the aromatic ring that acts as a spacer from the receptor moiety. The aromatic ring includes a second substituent in the *para* position that is a strong electron-donor (amino, hydroxyl or ether group, usually) and in the absence of the analyte inhibits fluorescent emission. The receptor system, indicated by dashed lines in Scheme 18, can have other groups with lone electron pairs, and/or suitable coordination geometry that increase selectivity to the target of interest. When complexation with the analyte is produced, electron transmission to the fluorophore is disconnected, and then it shows its fluorescent properties.

Moreover, their versatile chemistry allows the core derivatization in a variety of ways[75–77] leading to the generation of a multitude of chemosensors selectively to a wide range of metal cations of biological and environmental interest.[78] The attachment of adequate residues in several positions of the BODIPY core allows the building of sensors with specific spectroscopic and photophysical properties.[79]

To the best of our knowledge, Table 3 contains the properties of the BODIPY-based chemosensors able to detect Hg^{2+} following a PET mechanism, although in some cases, an intramolecular charge transfer (ICT) mechanism also exists, some of them proving to be very effective in concrete analytical applications. To see their chemical structures, please refer to Fig. 2.

A chemosensor containing a thiacalix[4](*N*-phenylazacrown-5)ether ligand linked by a spacer to a BODIPY core was described by Bitter and coworkers (BDP1, in Table 3).[79] Although the binding of Hg^{2+} ions results in an emission enhancement, the pronounced off–on response due to the PET process is generated in the presence of Cu^{2+}, and Fe^{3+} to a lower extent. In the presence of Ag^{+}, the latter response is diminished. No application to real samples was reported.

A BODIPY derivative with an *o*-aminophenol chelator at the *meso*-position which acts as a specific fluorescence turn-on sensor for Hg^{2+} and a selective colorimetric chemosensor for Cu^{2+} has been reported (BDP2, in Table 3).[74] After the addition of Hg^{2+}, the low quantum yield ($\varnothing < 0.002$) is increased to 0.01, with no interference of a wide variety of other ions. No application to real samples was reported to date.

M. Yuan *et al.* have reported the synthesis and sensing characteristics of a new class of colorimetric and fluorimetric dual-channel assay for the specific detection of Hg^{2+} in the presence of other cations (BDP3, in Table 3) that comprises both PET and ICT processes in a single molecule. In the absence of Hg^{2+} the BDP3 molecule is non fluorescent due to an efficient PET process. The latter is modified after the addition of Hg^{2+}, leading to an increment of the fluorescence intensity of *ca.* 7-folds.[80] No application to real samples was reported.

Du and coworkers have synthesized and characterized a new highly selective fluorescent sensor with an open-chain azadioxadithia (NO_2S_2) chelator for Hg^{2+} determination (BDP4, in Table 3). Without Hg^{2+} it has a low $\varnothing$, due to an efficient reductive PET from the NO_2S_2 chelator to BODIPY. A large fluorescent enhancement (160-fold) is produced after the addition of Hg^{2+} caused by the inhibition of the PET mechanism. Because of the great coordinating ability of Cl^{-}, Br^{-}, CO_3^{2-}, SCN^{-} and $CH_3CO_2^{-}$ to Hg^{2+}, the bright fluorescence of the Hg^{2+}–sensor complex is affected by these anions.[81] No application to real samples was reported.

A fluorescent chemosensor which exhibits selective fluorescence towards Hg^{2+} was presented by Fan and collaborators (BDP5, in Table 3). In contrast to sensor BDP4, it is unaffected by the presence of anions existing in its environment and organisms. By examining the application of BDP5 to PC12 cultured cells, it was observed that BDP5 could not permeate through the cell membrane. According to that, a BDP5-ester derivative nontoxic to cell cultures was used to get into cells and be transformed in BDP5 by esterase in the cytosol. The latter modification allows BDP5 to image intracellular Hg^{2+} in living cells, providing a useful way to study its toxicity and bioactivity.[82]

Lu *et al.* reported a fluorescent turn-on chemosensor able to selectively determine Hg^{2+} not only in aqueous media, but also within living cells (BDP6, in Table 3). In aqueous media, this sensor presents high sensitivity towards Hg^{2+}, reaching detection limits of $\leq$2 ng mL^{-1}, which is the U.S. Environmental Protection Agency's limit for drinking water.[83,84] Regarding living cells, experiments were conducted in HeLa cells by incubating them with BDP6 after addition of Hg^{2+}. Fluorescence determination with laser scanning confocal microscopy showed a significant fluorescence emission from the perinuclear region of the cytosol, indicating that BDP6 was cell-permeable, could respond to variations in intracellular Hg^{2+} and would be used to monitor Hg^{2+} and study its effects within living cells.[84]

Table 3 Spectroscopic and analytical parameters of the BODIPY-based chemosensors able to detect Hg^{2+} following a PET mechanism

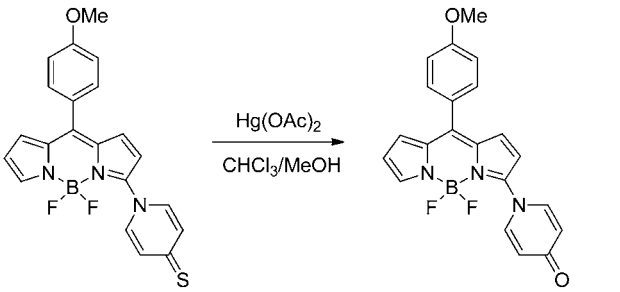
Sensor compound	∅	[R] (μM)	Reaction medium	λ_{ex} (nm)	λ_{em} (nm)	LOD (ng mL^{-1})	Interferences	R : Hg^{2+} stoichiometry	Mechanism	Analytical applications	Ref.
BDP1	0.062	10	CH_3CN	490	529	—	Cu^{2+}, Fe^{3+}, Ag^+	1 : 1	PET	—	79
BDP2	0.01	5	DMSO : HEPES buffer 1 : 99 (v/v), pH = 7.2	483	510	—	—	2 : 1	PET	—	74
BDP3	0.33	2	THF : HEPES buffer 30 : 70 (v/v), pH = 7.2	540	578	—	—	1 : 1	PET and ICT	—	80
BDP4	0.58	1	H_2O : CH_3CH_2OH, 7 : 3 (v/v), pH = 7.0	490	512	—	Ag^+, Cl^-, Br^-, CO_3^{2-}, SCN^-, $CH_3CO_2^-$	1 : 1	PET	—	81
BDP5	0.29	2	HEPES buffer, pH = 7.2	480	507	15	Ag^+, Pb^{2+}, Cu^{2+}	1 : 1	PET	Living cells	82
BDP6	0.054	0.5	DMSO : HEPES buffer 1 : 99 (v/v), pH = 7.2	470	509	2	Ag^+, Pb^{2+}	1 : 2	PET	Living cells	84
BDP7	0.13	5	CH_3OH	483	513	—	Al^{3+}, Fe^{2+}	—	PET	—	85
BDP8a, BDP8b	0.19, 0.61	2	Phosphate (0.1 M) solution, pH = 7.5	527, 527	538, 541	2	—	1 : 1, 1 : 2	PET, PET	—	86
BDP9	—	0.5	H_2O : CH_3CN, 60 : 40 (v/v)	498	507	—	Ag^+	1 : 1	PET	—	88
BDP10	—	10	CH_3CH_2OH : HEPES buffer (20 mM HEPES, 100 mM $NaNO_3$, 1 : 1(v/v), pH = 7.0)	495	513	≤2	Ag^+, Cu^{2+}	1 : 1	PET	Natural waters	89
BDP11	0.035	30	CH_3OH	492	520	5.6×10^2	Co^{2+}, Fe^{3+}	1 : 1	PET	Living cells	90

Lu and collaborators have developed a BODIPY-based fluorescence probe highly selective to Hg^{2+} in methanol (BDP7, in Table 3). The theoretical and experimental characterizations of the turn-on response in the presence of Hg^{2+} reveal that the enhancement is due to a prohibited reductive and oxidative PET processes, which allow the fluorophore to emit a strong fluorescence.[85] No application to real samples was reported.

Three fluorescent sensor molecules with polyamide receptors were designed and synthesized by Wang and collaborators, two of them having high sensitivity towards Hg^{2+}. The very weak basal fluorescence of BDP8a and BDP8b is largely enhanced after the addition of Hg^{2+}, because the reductive PET quenching process is impeded due to the positively cooperative metal–sensor complexation. The 2 ng mL^{-1} of Hg^{2+} detected using the molecule with the tetraamide receptor, in addition to the sensor's high water solubility, allow the development of probes useful to monitor low levels of Hg^{2+} in drinking waters.[86] The tetramide substituents act as electron donors, and upon excitation of the fluorophore, an electron transfer occurs; then the fluorescence is diminished when the fluorescent group in its excited-state is reduced. In this situation, the fluorescent group is acting as an acceptor and displays very weak fluorescence, resulting from the efficient PET quenching process from the electron-donating receptor moiety to the excited BODIPY fluorophore. In the presence of Hg^{2+} binding to the recognition tetramide moiety where the donor atom is present, the PET quenching pathway is efficiently blocked and an OFF–ON enhancement of the fluorescence is produced. The clear emission turn-on response is observed when concentrations as low as ng mL^{-1} level of Hg^{2+} are present and the reported molecular probe is able to act as an efficient and selective chemosensor unit for environmental Hg^{2+} monitoring. The high selectivity of the method towards other metal ions was corroborated by an interference study and the chemosensor unit is of potential application as a fluorescence sensor intended for *in situ* analysis by appropriate immobilization.[87]

A BODIPY appended thiacrown molecule able to detect Hg^{2+} and Ag^+ in a H_2O : CH_3CN medium has been reported (BDP9, in Table 3). The involved PET mechanism is blocked in the presence of Hg^{2+}, which interacts with the sulfur atoms of the thiacrown part.[88]

Fan *et al.* have developed a BODIPY derivative turn-on sensor able to sensitively and selectively detect Hg^{2+} even in the presence of cysteine or in sulfur-rich environments, which are known to form stable complexes with Hg^{2+} (BDP10, in Table 3). The aminophenol ligand added to the BODIPY is responsible



Scheme 19 Induced desulfuration reaction of BDP12 in the presence of Hg^{2+}.

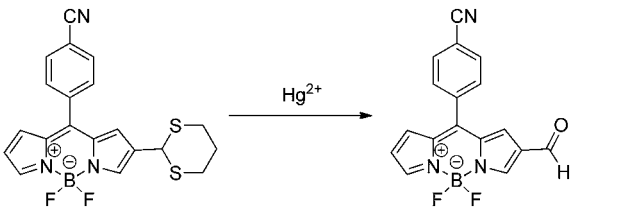
for the efficient PET process ($\varnothing = 0.3\%$), which reverts in the presence of Hg^{2+} with a 20-fold fluorescence intensity increase. This probe was successfully applied to the quantitation of Hg^{2+} at low levels in different kinds of natural water samples.[89]

A new chemosensor capable of selectively detecting Hg^{2+} over competing metal ions was designed by Vedamalai and Wu (BDP11, in Table 3). After the chelation of Hg^{2+} through the triazole units, the PET mechanism is blocked, leading to a great fluorescence enhancement. Experiments in HeLa cells were carried out in order to sense Hg^{2+} ions in living cells. Images gathered with a fluorescence microscope showed that the fluorescent signal was located in the intracellular area, which indicates a subcellular distribution of Hg^{2+} and a good permeability of BDP11.[90]

4.3 BODIPY based chemodosimeters

Khan and Ravikanth reported a 3-(pyridine-4-thione)BODIPY (BDP12 in Scheme 19) which can be used as a selective but not sensible, *i.e.* limit of detection of 3×10^6 ng L^{-1}, chemodosimeter for detecting Hg^{2+}. The latter was designed taking into account the great affinity between Hg^{2+} and sulfur, which allows a desulfurization reaction that is traduced in significant changes in the fluorescent properties of the chemodosimeter. In fact, the very low emission signal of BDP12 is increased after the addition of Hg^{2+} ions due to its desulfurization.[91]

A dual channel chemodosimeter for Hg^{2+} and Ag^+ detection was developed by Zhang and collaborators based on the concept of aldehyde group protection/deprotection. In the absence of Hg^{2+} the emission fluorescence of BODIPY-1,3-dithiane (BDP13 in Scheme 20) is very weak due to the PET caused by the sulfur on the thioacetal moiety. After the addition of Hg^{2+} a deprotection reaction of thioacetal occurs, which allows the enhancement of the basal fluorescence of BDP13 with a blue shift in the emission maximum and limit of detection of



Scheme 20 Dethioacetalization reaction of BDP13 in the presence of Hg^{2+}.

2.8×10^7 ng L^{-1}. On the other hand, Ag^+ did not desulfurize the thioacetal of BDP13. Instead of that, it coordinates with the sulfur atoms of the thioacetal moiety inhibiting the PET quenching of fluorescence and leading to the restoration of the emission without wavelength shift. Thus, this constitutes a BODIPY-based probe with dual channel fluorescence for two different metal ions.[92]

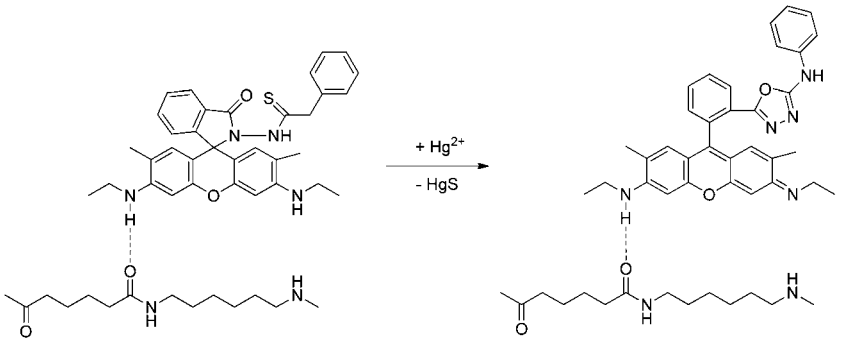
5 Immobilization of molecular probes in solid supports

A new trend in chemical sensing is based on the use of new materials for molecular probe immobilization. Polymers, crystals, glasses, particles, gold nanoparticles and nanostructures have made a profound impact on modern sensory systems, and the design and synthesis of novel materials with controlled sensing properties is a significant challenge within nanoscience and nanotechnology. However, there are only a few recent examples that concern Hg^{2+} detection.[93]

In a recent paper, double hydrophilic block co-polymers bearing rhodamine B-based Hg^{2+} reactive moieties were reported, which can serve as multifunctional sensors to pH, temperature and Hg^{2+} ions.[94] The block was composed of poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide-*co*-RhBHA) (PEO-*b*-P(NIPAM-*co*-RhBHA)). Non-fluorescent RhBHA moieties were subjected to selective ring-opening reaction upon addition of Hg^{2+} ions and pH, producing highly fluorescent acyclic species. Upon heating above the lower critical solution temperature (36 °C) of the PNIPAM block, they self-assemble into micelles possessing P(NIPAM-*co*-RhBHA) cores and well-solvated PEO coronas. It was found that the detection sensitivity to Hg^{2+} ions and pH could be dramatically improved at elevated temperatures due to fluorescence enhancement of RhBHA residues in the acyclic form, which were embedded within hydrophobic cores of the thermo-induced micellar aggregates.

In a second report,[95] novel fluorescent silica nanoparticles, formed by quantum dot SiO_2-RH 6G nanoparticles, with high selectivity towards Hg^{2+}, were synthesized for the detection of Hg^{2+}. The mercury ions promote the ring opening of spirolactam in the rhodamine moiety grafted onto the silica nanoparticles, resulting in a change in the fluorescence intensity. In this process, a Hg^{2+} ion takes part in the complexation with an oxygen atom of the rhodamine carbonyl group, resulting in the enhancement of the fluorescence intensity at 545 nm. When KI is added, the opened spirolactam is closed again, which can be attributed to the stronger binding ability of I^- than Hg^{2+}. A LOD of 0.52 ng mL^{-1} was reported with a linear range from 8.0 to 160 ng mL^{-1}, and the proposed approach was successfully applied to the determination of Hg^{2+} in tap and lake water samples with satisfactory results.

Finally, a highly sensitive and selective probe, sensor RG4 (ref. 44 and 45) (see Table 1), was proposed for the fluorimetric determination of mercury ion traces in aqueous solutions. The probe was based on the mercury-promoted ring opening of the spirolactam moiety of a rhodamine 6G spirocyclicphenylthiosemicarbazide derivative, retained in nylon membranes (Scheme 21). It was demonstrated that the chemodosimeter



Scheme 21 Proposed mechanism of Hg^{2+}-induced ring opening and cyclization of RG4 retained in nylon membranes.

preserves its sensor ability, displaying intense fluorescence in the presence of Hg^{2+} after being immobilized on the nylon surface and reacting with the mercury ion solution *via* a simple syringe procedure. The advantages of this proposal are: (1) the use of an easily affordable solid support which is able to immobilize the molecular probe without involving a covalent bond, (2) the consumption of a very small volume of organic reagent, dramatically reducing the environmental impact, and (3) the development of a solid phase system potentially useful as a main component for designing chemical sensors capable of providing continuous real-time information. Thus, a simple and sensitive fluorescence method for the determination of mercury ions was established. The limit of detection was 0.4 ng mL^{-1} (lower than the toxic levels in drinking water for human consumption, established by several regulatory agencies), and the sampling rate was about 15 samples per hour. The study of the potential interference from common cations demonstrated a remarkable selectivity for the investigated metal ions. The viability of determining Hg^{2+} residues in real water samples was successfully evaluated through the recovery study of several environmental water samples from different locations.

The same rhodamine derivative, sensor RG4, was also used to develop a novel Hg^{2+} fluorescent polymeric sensor phase to measure Hg^{2+} in solution, with high sensitivity and selectivity, in a quick and easy way.[46] The co-polymers were synthesized by the reverse ATRP technique, based on the copolymerization of methylmethacrylate (MMA) and HEMA, following a previously reported procedure.[96] To prepare the optimal sensing film, a mixture of co-polymer, plasticizer, and RG4 dye was prepared in acetone : methanol (80 : 20 v/v). The Hg^{2+}-sensitive sensing film was made by drop coating on poly(ethylene terephthalate) and was successfully applied for Hg^{2+} monitoring, showing high selectivity and relatively low response times (less than 30 minutes).

6 Conclusions

The development of efficient, selective and sensitive methods for monitoring Hg^{2+}, in environmental and biological systems, has been stimulated due to the alarm for the worldwide spread of mercury pollution and its damage to human health and the environment. A number of highly selective and sensitive fluorescent chemodosimeters and chemosensors, using rhodamine and BODIPY derivatives, have been exploited for *in vivo* monitoring of Hg^{2+} and for the detection of Hg^{2+} in environmental samples. Several limitations such as low water solubility, poor selectivity toward other metal ions, and weak sensitivity in physiological conditions prevent the sensor molecules being applicable in biological systems in some cases. Only a few of the above reported works discussed analytical figures of merit and few fluorescent probes work in pure aqueous solutions, and a number of the reported probes have not been applied in real samples. The summarized reports are conclusive of being a very active area of research and that there is a need for working on the design and application of fluorescent probes that may work in pure aqueous solutions at physiological pH. Development of such sensors will help to solve many problems of trace-level toxic Hg^{2+} determination, in different biological and environmental samples, in a simple and rapid way. The combination of Hg^{2+} responsive polymers and other immobilization structures and nanostructures, with well developed small molecules based on selective sensing moieties, are expected to exhibit preferred advantages, including enhanced detection sensitivity, biocompatibility, facile integration into devices and the ability of further functionalization for targeted delivery and imaging. The preparation of nanostructured and/or polymeric sensing films, with reduced response times, in combination with optical fibers, is a fundamental step for the development of practical sensor optrodes, applicable for real time and *in situ* monitoring.

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