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COMMUNICATION

A fluorescent ratiometric nanosized system for the determination of Pd^{II} in water†

Cite this: DOI: 10.1039/x0xx00000x

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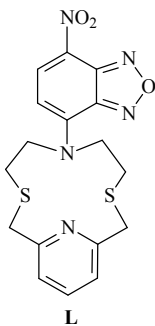
Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

The fluorescent ligand (L) based on N2S2 pyridinophane macrocycle and the 7-nitrobenzo[1,2,5]oxadiazole (NBD) fluorogenic fragment has been synthesized to coordinate Pd^{II} ions. Loaded on dye-doped silica nanoparticles, L can be used as ratiometric fluorescent chemosensor for Pd^{II} in water with high selectivity toward other metal ions including the platinum group ones.

The outstanding properties of platinum group metals (PGM) as the high resistance to oxidation, the catalytic behavior and recyclability are at the basis of their increasing use.¹ They are essential in a broad range of applications, spanning from automotive industry, industrial catalysis, fuel cells, super alloys up to medicine. Among PGM, palladium and its compounds find rising applications in exhaust systems to reduce the emission of gaseous pollutants and as efficient catalysts in drug synthesis.² In this case, the removal process of palladium from the final product is extremely important due to its toxicity since it can be transported into biological tissues and its accumulation can constitute a potential health hazard. In the case of exhaust systems, an efficient and complete recovering of palladium has, instead, a high economic impact due to its cost.³ The development of efficient methods for the analysis of trace Pd^{II} content with specific selectivity over other PGM group ions is therefore a very important issue and a very challenging task. In the last years several systems for sensing Pd^{II} have been synthesized, many of them working as chemodosimeters.⁴ Fluorescent chemosensors based on a pure ligand-metal interaction are quite rare and they work in mixed aqueous/organic media.⁵ To date, we are not aware of fluorescent system able to sense Pd^{II} in pure water. In this communication we report the coordinating and fluorescence properties of the new chemosensor **L** (see ESI† for synthetic details) containing the fluorescent fragment 7-nitrobenzo[1,2,5]oxadiazole (NBD) linked to the 1,7-diaza-4,10-dithia-2,6-pyridinophane macrocycle, in different solvents including water. Since Pd^{II} is a highly thiophilic metal ion, a sulphur-based ligand moiety can be successfully employed. We have first



investigated the sensing properties of **L** in acetonitrile (AN), in DMF, in AN/water 1:1 and in DMF/water 4:1 (v/v) solvent mixtures. In DMF the absorption spectrum of **L** shows the two typical bands due to the 4,7-disubstituted benzofurazan moiety at 340 nm, associated with the π - π^* transition, and at 490 nm attributed to intramolecular charge transfer transitions (CT). In this solvent, free **L** is highly fluorescent (λ_{em} =540 nm, λ_{ex} =340 nm Φ_{em} =0,16). A quite similar behavior was observed in AN (Φ_{em} =0,10).

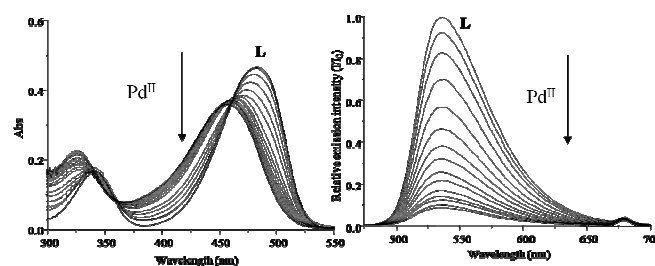


Figure 1. UV-Vis and fluorescence (λ_{ex} = 340 nm) titration of **L** with K_2PdCl_4 in DMF/water 4:1 (v/v) at 298 K ($[L] = 1.5 \times 10^{-5}$ M, $[K_2PdCl_4] = 0 \rightarrow 1.5 \times 10^{-4}$ M).

The addition of K_2PdCl_4 affects both the UV-vis and fluorescence spectra of **L**. In particular, the addition of Pd^{II} causes a blue shift of the absorption band of the free ligand at 490 nm to 450 nm, together with the quenching of the fluorescence band (data not shown). The same behavior was observed in AN (data not shown). However, since the removal processes of palladium after its industrial use should be done in water for environmental and solubility reasons, we wanted to check the optical response of **L** in solvent media closer to the application conditions, and, therefore, further studies were performed in AN/water 1:1 and in DMF/water 4:1 (v/v) mixtures, where the ligand and its metal complexes are still highly soluble. Spectrophotometric and spectrofluorimetric titrations with increasing amounts of Pd^{II} of a solution of **L** in DMF/water (4:1 v/v) (Figure 1) indicated the formation of a 1:1 complex ($\log K = 6.06$). The fluorescence emission of a solution containing 10^{-5} M of **L** is completely quenched in the presence of 5 ppm of palladium (5 eq.). The stoichiometry of the mononuclear complex was determined

to be PdLCl₂ on the basis of the elemental analysis of the solid obtained from a saturate DMF solution (see ESI† for synthetic details). Competition experiments with Co^{II}, Cu^{II}, Ni^{II}, Cd^{II}, Pb^{II}, Hg^{II}, Zn^{II}, Ag^I and K₂PtCl₄ showed that the co-presence of other metal ions in addition to Pd^{II} cause negligible effects in the resultant emission intensity in DMF/water (4:1 v/v) of the Pd-complex (see ESI†, Figure S1). However, only the presence of Pd^{II} significantly quenches the fluorescence underlying that **L** is selective towards Pd^{II}. Analogous results were obtained in AN/water 1:1 (v/v).

In order to further characterize the Pd/**L** complex we performed ¹H-NMR titrations in dry DMF-*d*₇. By adding Pd^{II} ions, the relative intensity of the resonances due to free **L** progressively decreased, whereas those assigned to the 1:1 Pd-complex emerged in the spectrum. Further addition of Pd^{II} took to a gradual disappearing of these signals and to the appearing of a third series of resonances attributed to a 2:1 Pd/**L** complex (see Figure 2 for a stack plot of the methylenic region), which is not formed in DMF/water 4:1 (v/v) (see ESI† for a deeper discussion and for the distribution diagram of the three species in DMF, Figure S3). It is important to underline that, on the basis of ¹H-NMR spectra, the 1:1 species has the same structure in both dry DMF and DMF/water 4:1 (v/v) solvents, and that while in dry DMF the 2:1 Pd/**L** species can be observed, in the presence of water it does not form. The observation of resolved resonances for all the three species in the same spectra (at intermediate Pd^{II} concentrations) indicates that Pd exchange rate is slow with respect to the NMR time-scale, i.e. $k_{ex} \ll \Delta\nu$, where $\Delta\nu$ is the frequency difference between the resonances (17 s⁻¹ in the present case).

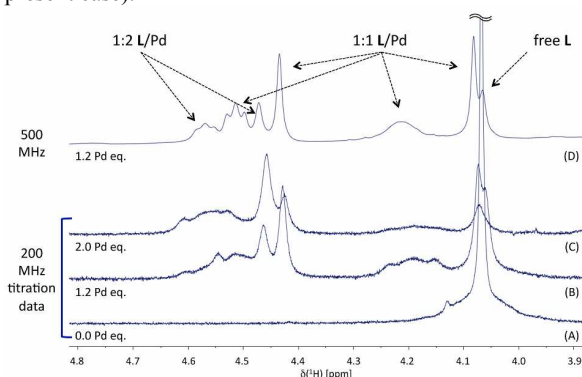


Figure 2. Stack plot of four selected ¹H-NMR spectra (A-C) in the 3.9-4.8 ppm range at different K₂PdCl₄ concentrations, in DMF-*d*₇. (see ESI† for the complete stack plot, Figure S3, and for a complete discussion).

These results show that, in the absence of Pd^{II} ions, the ligand is symmetric from the NMR point of view, i.e. the homologous methylenes on the two side of the macrocycle are magnetically equivalent. Upon formation of the 1:1 complex, the homologous methylenes lose their equivalence (see ESI† for details, Figures S2, S4). Finally, symmetry was regained in the 2:1 Pd/**L** complex, as shown by a unique resonance for each type of methylenic group.

On the basis of ¹H¹³C-gHSQC (ESI†, Figures S5, Table S1), dipolar through-space correlations found in the homonuclear NOESY spectra (ESI†, Figure S6), and by Hybrid-DFT and TD-DFT calculations also accounting for solvation effects (see ESI† for details, Figure S7, Tables S2-S8), we could demonstrate that the asymmetry of the 1:1 complex is compatible with the structure reported in Figure 3 for a mononuclear PdLCl₂ species. The metal ion is in a pseudo-square planar coordination geometry achieved by the N atom of the macrocycle and one neighboring sulphur atom. No interaction with the N atoms of the pyridine ring or the benzofuran pendant is possible due to geometry constraint.

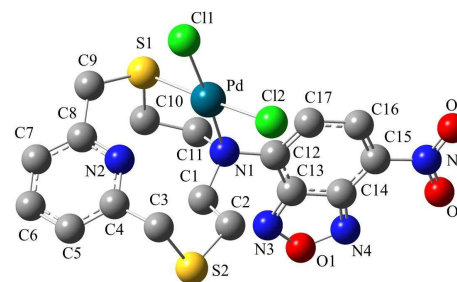


Figure 3. Ball and stick drawing and atom labelling scheme of the structure of the complex PdLCl₂ optimized at DFT level in DMF. Hydrogen atoms have been omitted for clarity.[‡]

As mentioned, however, most of the possible sensing applications of this species could be in pure water and, due to the very poor solubility of **L** in this solvent, we decided to use silica core-polyethylene glycol (PEG) shell nanoparticles (NPs) as vehicles and incubators to host **L** and favor the Pd^{II} binding events in water.^{6, 7} Since a few years, we are actively developing, and satisfactorily applying in many fields, very stable and highly water soluble nanoparticles presenting a hard silica core of 10 nm of diameter surrounded by a soft PEG shell 7 nm thick, for a total average hydrodynamic diameter of ca. 25 nm.⁸ Due to their synthetic procedure based on the inverse micelle method, they are very monodispersed (ESI† Figure S8) and they do not undergo aggregation and precipitation in water even after several months. Moreover all the preliminary results that we have obtained so far⁸ are in favor of their benign nature thanks to their stability also in physiological conditions, their low non-specific interactions and to the fact that the external shell is made of a stealth polymer. This last point is of fundamental importance for their use as vehicular: the soft and polymeric structure of this outer shell permits to embed poorly water soluble molecules and transport them in water. Their location in the NP preserves their possibility to interact with the external water environment but, very interestingly, can also allow their communication with species present in the core.^{9, 10} It is in fact possible to copolymerize opportunely silanized fluorophores in the inner part of the NPs during the growing, to prevent their leaking and to obtain particles with different doping degrees.¹¹

We have, therefore, prepared 3 mL of a 1.25×10^{-7} M solution of NPs in pure water and added increasing amounts of a 4.75×10^{-4} M solution of **L** in AN. We chose to add the ligand from a solution and not as a solid as already experimented⁹ to gain control on the concentration and to avoid the formation of pre-aggregates but the maximum amount of AN present in the final solution is always lower than 0.5%. We have first used dye-doped nanoparticles, that is to say NPs bearing a dye (silanized DEAC:7-(diethylamino)-2-oxo-N-(3-(triethoxysilyl)propyl)-2H-chromene-3-carboxamide) covalently linked inside the silica core to prove the effective inclusion of **L** in the NPs. The photophysical properties of **L** and DEAC, in fact, are suitable to induce energy transfer from DEAC (donor) to **L** (acceptor) when the two partners are positioned in close proximity and the DEAC is selectively excited. The inclusion of the ligand in the NP provides the right distance for the energy transfer to occur.^{9, 10} The addition of **L** (from 1 to 35 equivalents) to the NP solution was photophysically monitored (ESI†, Figures S9-S10) and the changes in the absorption spectra evidenced the rise of a band at ca. 500 nm, the typical one of **L** with a red shift that can be realistically attributed to the different environment.

Assuming as a reasonable approximation that the molar extinction coefficient measured in AN/water 1:1 (v/v) ($\epsilon_{490} = 3.3 \times 10^4$ M⁻¹ cm⁻¹) could be used also for the molecules included in the DEAC@NPs,

the evolution of the absorption spectra revealed that the maximum number of ligand moieties allocated per nanoparticle is ca. 30 (ESI Figure S11). The emission spectra evidenced the decrease of the band centered at 464 nm (typical of DEAC) and the concomitant increase of that at 528 nm (typical of **L**), by exciting at 400 nm where the absorption of **L** is negligible in comparison with that of DEAC. This clearly indicates the occurring of an efficient (ca. 60%, ESI† Figure S10) energy transfer proving the close proximity of the chemosensor to the doped core.

To investigate the response of **L** to the presence of Pd^{II} ions in water we decided to use a 20:1 (**L**:DEAC@NPs) ratio in order to obtain a system in average conditions. The addition of increasing amounts of K₂PdCl₄ induces changes in the absorption spectra and a decrease of the emission band at 528 nm that indicates the complexation of the metal ions by **L** also in water in the presence of DEAC@NPs (Figure 4).

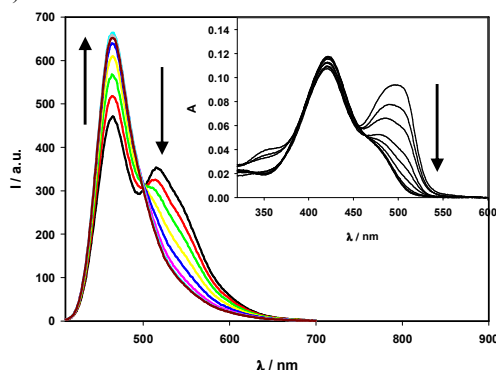


Figure 4. Emission spectra of 1.25×10^{-7} M **L**:DEAC@NPs 20:1 in water ($\lambda_{\text{ex}}=400$ nm) upon addition of increasing amounts of K₂PdCl₄ 4×10^{-4} M in AN; inset: absorption spectra of the same titration.

The addition of one equivalent of the other investigated metal ions as nitrates (or perchlorate), to **L** in water in the presence of DEAC-doped nanoparticles did not cause any change either in the absorption or in the emission spectra. The response of the Pd complexed system to the addition of 10 equivalents of each of them is also negligible and it is very similar to that obtained in organic/water mixed solvents. Changes in the luminescence of **L** were observed only in the presence of an excess of Hg^{II} ions (ESI† Figure S12).

Analogous results have been obtained with non-doped NPs. The initial absorption and emission spectra of **L** have the same shape of the ones in AN/water solution, with only a small blue-shift, and Pd(II) complexation causes the same changes in the absorption and emission (ESI† Figure S13) spectra already discussed in solution taking to a complete quenching of **L**. In the measurements in pure water (in presence of NPs) we did not observe any kinetic effect and therefore it was not necessary any equilibration time before the acquisition of the electronic spectra. The only cation that shows a slow coordination kinetic was Pt^{II} in absence of palladium. In Pd^{II}/Pt^{II} competitive experiments, however, the observed spectral changes were the same ascribable to only palladium and they were stable in time (Figure S12), highlighting the selectivity of the system towards Pd^{II} over Pt^{II}.

By plotting the emission versus the added amount of metal ion, we could calculate an association constant value of $\log K=7.86$ for the formation of a 1:1 complex in water (ESI† Figure S14 for the Job's plot), a value and a behavior similar to those observed in AN/water and DMF/water.

In conclusion, we have synthesized a new fluorescent ligand able to detect selectively the Pd^{II} ion in organic/aqueous mixed solvent via CHEQ effect of the fluorescence emission. Loaded on fluorescent

DEAC-doped silica nanoparticles, **L** can be used as ratiometric fluorescent chemosensor for Pd^{II} ions in aqueous solution with high selectivity over other PGM metal ions. This is the first example of a chemical system able to selectively detect Pd^{II} ions in pure aqueous medium.

We acknowledge financial support from the Universities of Urbino, Cagliari and Bologna (FARB Project Advanced Ultrasensitive Multiplex Diagnostic Systems Based on Luminescence Techniques) and Leonardo Neri for performing some fluorescence experiments.

Notes and references

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†Electronic Supplementary Information (ESI) as noted in the text are available. See DOI: 10.1039/c000000x/

‡Selected bond lengths, angles, and dihedrals: Pd-C11, 2.308; Pd-C12, 2.333; Pd-N1, 2.259; Pd-S1, 2.327 Å; C11-Pd-C12, 90.35; S1-Pd-N1, 86.40; C11-Pd-S1, 85.15; C12-Pd-N1, 98.10°; C11-C12-S1-N1, 177.82; C2-N1-C12-C13 49.27°.

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