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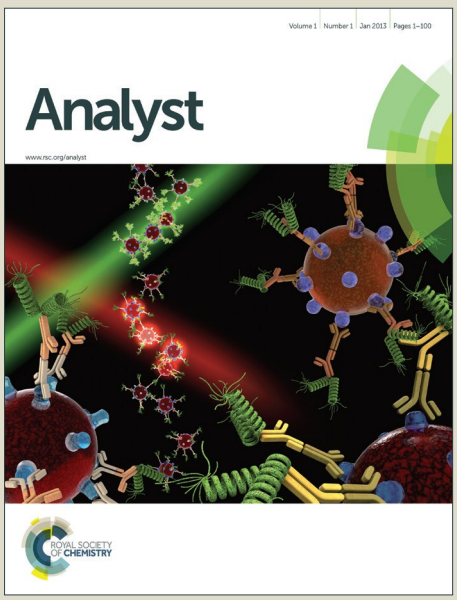

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Fluorescent and Colorimetric Probe Containing Oxime-Ether for Pd^{2+} in Pure Water and Living Cells

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Palladium contamination has potential danger in the environment, and the biological safety study is still on the way. Here, a fluorescent and colorimetric probe (compound 1) containing oxime-ether for Pd^{2+} has been developed. 1 can detect Pd^{2+} in aqueous samples with high selectivity and sensitivity. 1 can also works well in biological samples, which give a choice to value Pd^{2+} in an organism.

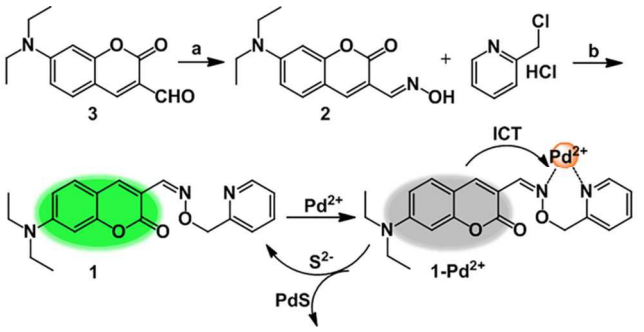
As one of the most important noble metal catalysts, palladium is widely used in organic synthesis. A lot of organic reactions, such as cross-coupling reaction and carbonyl reaction can perform smoothly with palladium catalysts.[1] When palladium catalysts are applied widely, some problems occur at the same time. The palladium catalysts used in these organic reactions cannot be completely recovered, and it will be discharged into the environment with the wastewater. Palladium is also used widely in the vehicle exhaust catalyst systems to reduce emissions of gaseous pollutants such as CO and NO.[2] Therefore, Palladium emission from vehicle catalytic converters increase the contamination in the environment. The residue palladium in the environment will eventually appear in our diet through the food chain and may cause potential danger to human health. For instance, palladium may disturb a variety of cellular processes by binding to the thiol-containing amino acids, proteins, DNA, and other biomolecules.[3] A very low doses of Pd^{2+} can sufficiently cause allergic reactions in susceptible individuals.[4] Therefore, it is very meaningful to detect residual palladium in food, environment and living organisms.

Some well-known methods such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometer (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are used in metal element analysis, but they need expensive instruments with high maintenance costs, complex sample pretreatments and professional technicians.[5] Moreover, these methods are difficult to be used in biological samples. An optical (colorimetric and fluorescent) probe has the characteristics of good selectivity, high sensitivity and convenient operating.[6] Besides, it does not require expensive facilities and can be used in biological detection. Owing to the advantages of optical probe, some fluorescent and colorimetric probes for palladium have been developed in recent years. On the basis of the coordination ability of palladium with O, N, P, S and Se, the coordination type sensors are designed, such as azo compounds,[7] oxime compounds,[8] hydrazone compounds,[9] thioether compounds,[10] thiourea compounds,[11] dithiomaleonitrile compounds,[12] thiophene compounds,[13] selenadiazole compounds.[14] and so on. On the basis of the catalytic ability of palladium, the chemical reaction type sensors are explored, such as Pd-catalysed spiroring-opening of xanthenes,[15] Pd-catalysed cyclization of heterocyclic,[16] Pd-catalysed depropargylation,[17] Pd-catalysed Tsuji–Trost reaction,[18] Pd-catalysed coupling reaction[19], Pd-catalysed desulfurization[20] and so on. Although there are many probes for palladium are reported, most of them need to be operated in a mixture of water and organic solvent, and only few of them can be applied to do sensing in living cells. Therefore, the development of a practical probe for palladium ions remains an urgent job.

According to the previous literature reports,[8] probes based on oxime derivatives for Pd^{2+} have the characteristics of quick response and high sensitivity, but most of them can only detect Pd^{2+} by colorimetry, and the selectivity of them also needs to be improved. So we hope to further develop the oxime derivatives and design more excellent probe for Pd^{2+}. By introducing coumarin as a fluorophore and changing oxime to oxime-ether, we have developed a fluorescent and colorimetric probe containing oxime-ether (compound **1**). **1** can detect Pd^{2+} in aqueous samples, such as surface water and underground water, and it can also be applied to detect Pd^{2+} in living cells. Compared

with the reported sensors, oxime ether is applied to detect Pd^{2+} for the first time.

Probe **1** can be easily synthesized according to Scheme 1: coumarin aldehyde (compound **3**) reacted with hydroxylamine hydrochloride in EtOH at room temperature to form coumarin oxime (compound **2**). Then compound **2** and 2-(chloromethyl)pyridine hydrochloride was refluxed in MeCN to obtain compound **1.** Compound **1** was confirmed by ^1H NMR, ^{13}C NMR and HRMS (Fig. S1).



Scheme 1 Synthetic route of **1** and the proposed mechanism for **1** to identify Pd^{2+}. (a) hydroxylamine hydrochloride, K_2CO_3, EtOH, room temperature, 94%; (b) 2-(chloromethyl)pyridine hydrochloride, K_2CO_3, MeCN, reflux, 68%.

Compared with the reported insoluble sensors for Pd^{2+}, **1** can detect Pd^{2+} in pure water without the aid of any organic solvent. As shown in Fig. 1a, **1** has a strong green fluorescence in water with an emission peak at 505 nm. When Pd^{2+} was gradually added into the aqueous solution of **1** (10 μ M), the fluorescence intensity at 505 nm decreased consistently. It could be clearly observed by naked-eyes that the fluorescence of the solution changed from green to almost no fluorescence during the fluorescence titration procedure. When the concentration of Pd^{2+} achieved to 10 μ M, the fluorescence intensity of the solution at 505 nm decreased by about 96%. The fluorescence response of **1** towards Pd^{2+} was calculated to cover a linear range from 0 μ M to 6 μ M ($R^2 = 0.9909$), with the limitation of detection as low as 55 n M (Fig. S2). Compared to some previously reported fluorescent probes[15a,b,c,d] for Pd^{2+}, Probe **1** is not the most sensitive probe, one reason for this is that Probe **1** is a "turn off" type sensor (a "turn off" type sensor is usually less sensitive than a "turn on" one [2a]), another reason might be that probe **1** works in pure water rather than a mixture of water and organic solvent. But according to the WHO specified threshold limit for palladium content in drug chemicals [5.0 ppm to 10.0 ppm (47.0 μ M to 94.0 μ M)],[7] the sensitivity of **1** can satisfy the requirement. **1** can also detect Pd^{2+} in pure water by naked eyes. With the fluorescent changes of **1** from strong to weak in the titration procedure, the color of the solution has an obvious change from green to yellow, and this phenomenon is in accordance with the absorption of **1.** The peak at 425 nm decreased consistently while the absorption at 478 nm increased consistently upon the gradual addition of Pd^{2+} (Fig. 1b and Fig. S3). When 1.0 equiv. of Pd^{2+} was added to a water solution of **1** (10 μ M) one-time, the fluorescence of the solution was quenched almost completely within 30 seconds and remained stable within 30 minutes (Fig. S4), so **1** is an effective probe for Pd^{2+}. Finally, a pH investigation indicated that **1** could detect Pd^{2+} in a wide pH span of 4-10 (Fig. S5).

In order to investigate the selectivity of the probe toward Pd^{2+}, we researched the response of 22 kinds of cations with **1**. As showed in Fig. 2 and Fig. S6, among the 22 kinds cations, only Pd^{2+} could quench the fluorescence of **1** almost completely.

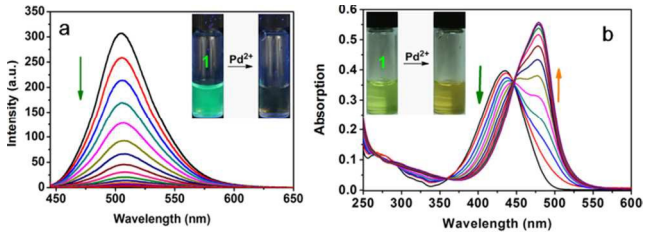


Fig. 1 (a) Fluorescence spectra of **1** (10 μ M) in water solution in response to the presence of Pd^{2+} (0.0 to 1.5 equiv.), λ_{ex} = 435 nm. Inset: fluorescence images of **1** and **1** + Pd^{2+}. (b) UV-Vis spectra of **1** (10 μ M) in response to Pd^{2+} (0.0 to 1.2 equiv.) in water solution. Inset: color images of **1** and **1** + Pd^{2+}.

Au^{3+} and Cu^{2+} had a certain quenching effect on the probe when 2.5 equiv. of them were added respectively. However, they did not show significant interference effect because the solution of **1** still had a strong green fluorescence as showed in Fig. S6. The rest of competitive cations including Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Na^+, Pb^{2+}, Pt^{2+}, Rh^{3+}, Zn^{2+} and Zr^{4+} (each of them was added by 5.0 equiv.) showed little or almost no interference. More importantly, even in the presence of the above-described competitive cations, Pd^{2+} still kept the similar fluorescence response. The selectivity of the probe toward Pd^{2+} can also be judged by the color change, as showed in Fig. S7, with the other competing cations only Pd^{2+} can cause an obvious color change (from green to yellow) of the water solution of **1**. All these results indicate that **1** is a highly selective probe for Pd^{2+}.

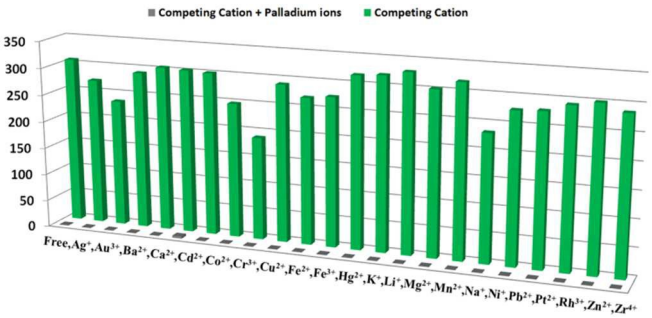


Fig. 2 Fluorescence intensity of **1** (10 μ M) in water solution at 505 nm after addition of various cations (green bars) and those after further addition of 1.0 equiv. of Pd^{2+} (black bars). Au^{3+} and Cu^{2+} were added by 2.5 equiv., other competing cations including Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Pt^{2+}, Rh^{3+}, Zn^{2+}, Zr^{4+} were added by 5.0 equiv. λ_{ex} = 435 nm.

Practical application ability is also an important factor to judge a probe. Three experiments are done to evaluate the practical application ability of **1**. In the first experiment, the actual water samples were used as a solvent instead of distilled water. As showed in Fig. S8, in the surface water and underground water, **1** could still work well as a sensor for Pd^{2+}. In the next experiment,

the living cells fluorescence imaging experiment was conducted to study whether **1** could penetrate the cell membrane to detect Pd^{2+} in living cells. As shown in Fig. 3, when Hela cells were incubated with **1** (10 μ M) for 30 min at 37 ºC, the cells showed strong green fluorescence (Fig3a, 3c). After treated with Pd^{2+} (10.0 μ M) for 15 min at 37 ºC, the cells showed almost no fluorescence (Fig. 3d, 3f). The changes of the images of Hela identified that **1** could probe Pd^{2+} in organism samples. Finally, the preliminary results of cytotoxicity assays of **1** show that 1 is not toxic to cells within the range of 0-100 μM. The results of these experiments indicate that as a probe for Pd^{2+}, **1** can work in a relatively complex environment and has the potential to detect Pd^{2+} in biological samples.

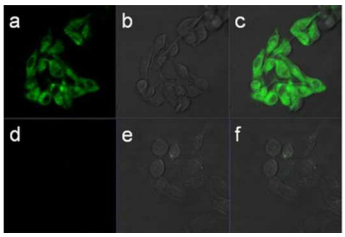


Fig. 3 Fluorescence images of Hela cells incubated by **1** (10 μ M) for 30 min at 37 ºC (a, from dark field; b, from bright field; c, merged image of a and b) and further treated with Pd^{2+} (10.0 μ M) for 15 min at 37 ºC (d, from dark field; e, from bright field; f, merged image of d and e).

For better understanding the detection mechanism of the probe, an reversible experiment was performed by adding 4.0 equiv. of S^{2-} into a solution of **1**-Pd^{2+} (10 μ M **1** + 10 μ M Pd^{2+}), it could be observed that the fluorescence of the solution recovered its original green fluorescence (the fluorescence intensity of the solution at 505 nm restored by 95.5%), accompanied with the color changed from yellow to green (Fig. S9a). The result of the reversible experiment indicated that the interaction between **1** and Pd^{2+} was on the basis of coordination effect. Then Job's-plot measurements were carried out to quantify the complexation ratio between **1** and Pd^{2+}. As shown in Fig. S9, UV-Vis measurements and fluorescence emission measurements all showed that the molar fraction of [Pd^{2+}] / [**1** + Pd^{2+}] was about 0.5, which indicates that the coordinated ratio between Pd^{2+} and **1** is about 1 : 1. The results of ^{1}H NMR and HRMS spectra provide additional evidences that **1** and Pd^{2+} can form a 1: 1 coordination compound (Fig. S1). Based on these results, we proposed the possible mechanisms of **1** detecting Pd^{2+} as shown in Scheme 1. When Pd^{2+} is added into the water solution of **1**, a 1:1 coordination complex with **1** is formed. Owing to the electron-withdrawing effect of palladium, an intramolecular charge transfer (ICT) effect within **1** is induced. As a result, the fluorescence of **1** is quenched, accompanied with a red shift of the maximum absorption wavelength (from 435 nm to 478 nm) of **1**. When S^{2-} is further added, it snatches Pd^{2+} from the **1**-Pd^{2+} complex and forms the more stable compound PdS. As a result, the fluorescence of the mixture solution recovers. So **1** is a reversible probe for Pd^{2+}.

In summary, by changing oxime to oxime-ether, a fluorescent and colorimetric probe for Pd^{2+} has been reported for the first time. Compared with the oxime derivatives probes for Pd^{2+}, the novel oxime-ether derivative (probe **1)** showed more excellent properties. **1** can detect Pd^{2+} in pure water with high selectivity and sensitivity. More importantly, **1** can be applied to detect Pd^{2+} in real water samples and living cells. We hope these results will contribute to the rational design of more excellent probe for Pd^{2+} in the future.

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Notes and references

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