Analyst

Cite this: *Analyst*, 2012, **137**, 2054

www.rsc.org/analyst

PAPER

One-step synthesis of fluorescein modified nano-carbon for Pd(II) detection *via* fluorescence quenching

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Received 16th December 2011, Accepted 2nd March 2012
DOI: 10.1039/c2an16261j

Carbon black (CB) nanoparticles modified with fluorescein, a highly fluorescent molecule, were prepared using a facile and efficient methodology. Simply stirring CB in aqueous solution containing fluorescein resulted in the strong physisorption of fluorescein onto the CB surface. The resulting Fluorescein/CB was then characterised by means of X-ray photoelectron spectroscopy (XPS), cyclic voltammetry (CV), fluorescence microscopy and fluorescence spectroscopy. The optimum experimental conditions for fluorescence of Fluorescein/CB *viz.* fluorescence excitation and emission wavelengths, O_2 removal and the amount of Fluorescein/CB used, were investigated. The Fluorescein/CB was used as a fluorescent probe for the sensitive detection of Pd(II) in water, based on fluorescence quenching. The results demonstrated that the fluorescence intensity of Fluorescein/CB decreased with increasing Pd(II) concentration, and the fluorescence quenching process could be described by the Stern–Volmer equation. The limit of detection (LOD) for the fluorescence quenching of Fluorescein/CB by Pd(II) in aqueous solution was found to be 1.07 μM (based on 3σ). Last, approaches were studied for the removal of Fe(III) which interferes with the fluorescence quenching of Fluorescein/CB. Complexation of Fe(III) with salicylic acid was used to enhance and control the selectivity of Fluorescein/CB sensor towards Pd(II) in the presence of Fe(III).

1. Introduction

Palladium is widely used in various catalytic reactions in industry, such as in hydrogenation of unsaturated hydrocarbons,[1] as well as in automobile catalytic converters, fuel cells, jewellery and dental crowns.[2–7] Most importantly, it plays an important role in synthetic organic chemistry as a catalyst.[2,3] However, a high level of residual palladium is often found to get into the environment through the waste water system, typically due to leaching from catalysts used in synthetic protocols, resulting in a devastating impact upon the health and well-being of both humans and wildlife.[2–5] According to the recommendations from the European Agency for the Evaluation of Medicinal Products (EMEA) for the allowable level of metals in drugs, an oral concentration limit for palladium should be $\leq$5 ppm (*ca.* 4.7×10^{-5} M).[4,8] Therefore, it is important to develop efficient methods to detect palladium selectively and sensitively.

Fluorescence spectroscopy is an extremely sensitive analytical technique, which has recently been proposed for palladium(II) analysis.[4,5] Moreover, much attention has been given to the synthesis of fluorescent sensors for the detection of heavy metal ions in biological and environmental samples, owing to the selective, sensitive, non-destructive and fast nature of their emission signals.[9–12] For example, it has been reported in the literature that a variety of highly fluorescent chromophore-functionalised nanomaterials for metal ions sensing have been synthesised *via* covalent and non-covalent modifications.[12–15] In particular, functionalised carbon nanomaterials which exhibit visible fluorescence emissions were prepared by coupling carbon nanomaterials to different fluorophores, such as fluorescein.[12–15]

Fluorescein is a highly fluorescent molecule, widely used for labelling membranes and proteins.[16–18] At different pH values, fluorescein appears in its various forms: cation, neutral molecule, monoanion and dianion (as shown in Fig. 1), making its absorption and fluorescence properties strongly dependent upon pH.[18–23] In strongly acidic solutions, the cation is the predominate species. At pH values from 2 to 4, fluorescein exists in its neutral species, and as the pH values become more basic, within the range of 4.3 to 6.4, the monoanionic form is present. At pH above 6.4, the dianion is the most prevalent.[18–23] Note that the strong fluorescence of fluorescein appears in solutions of pH greater than 6.5; lowering the pH results in a decrease in the fluorescence intensity.[18,19]

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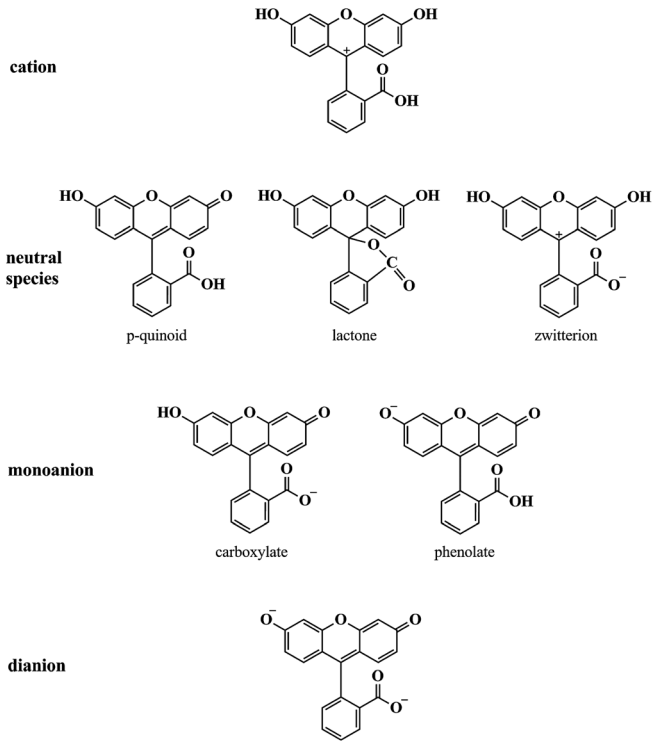


Fig. 1 Chemical structures of Fluorescein at different pH values of solution.

Carbon-based materials such as glassy carbon (GC), carbon nanotube (CNT) and graphite are widely used as solid supports in several modifications due to their rich surface chemistry, chemical inertness, and suitability for various sensing and detection applications.[24,25] However, such materials are still relatively expensive.[26,27] Graphite is a significantly cheaper alternative, although it possesses relatively poor surface area-to-volume ratios when compared to nanomaterials.[26,27] Carbon black (CB) nanoparticulate materials are therefore extremely favourable alternatives due to their significantly lower cost, widespread availability and very high surface area-to-volume ratios compared with those carbon substrates discussed earlier.[26–28] CB consists of primary particles with a quasi-graphitic microstructure and is commercially available in nanodimensions, *e.g.* of 14 nm in diameter or above.[27–29]

In this work, we report a novel analytical method based on fluorescence quenching for the detection of Pd(II) in aqueous solution by fluorescein-modified CB material (Fluorescein/CB). The synthesis of Fluorescein/CB was environmentally friendly, extremely simple and convenient with no hazardous chemicals involved. The physisorption of fluorescein on CB surface occurred when CB was stirred in fluorescein solution. The resulting Fluorescein/CB could selectively detect Pd(II) in aqueous solution with no Fe(III) interference provided the latter is complexed with salicylic acid. Other metals such as Cu(II) and Ni(II) will interfere by similar quenching of the fluorescence,[9] but the detection of palladium in the presence of iron is a particular challenge because of the need to measure palladium in waste water stream resulting from industrial plants using palladium reagents for organic synthesis. Such streams are likely to contain palladium and iron but not other metallic species.

2. Experimental

2.1 Reagents and equipments

The commercial Monarch® 430 carbon black (M 430), diameter 27 ± 10 nm, used in this study was kindly donated by James M Brown Ltd (Staffordshire, UK). Fluorescein was supplied by Sigma-Aldrich (Gillingham, UK). Salicylic acid, sodium salt (2-(HO)$C_6H_4CO_2Na$) was purchased from BDH (Poole, UK). Palladium(II) nitrate dihydrate ($Pd(NO_3)_2 \cdot 2H_2O$), ammonium iron(III) citrate ($C_6H_8O_7FeNH_3$), and all other chemicals were purchased from Sigma-Aldrich (Gillingham, UK). All the reagents were used without further purification. All solutions were prepared using deionised water of resistivity not less than 18.2 MΩ cm^{-1} at 298 ± 2 K (Millipore UHQ, Vivendi, UK).

Sonication was carried out using a D-78224 Singen/Htw sonicator (50/60 Hz, 80 W, UK). Centrifugation was carried out using a Centrifuge 5702 (Eppendorf, UK). pH measurement was made using a pH213 pH meter (Hanna instrument, UK). X-ray photoelectron spectroscopy (XPS) was performed on VG ESCALAB MkII at the Chemical Research Laboratory, Department of Chemistry, University of Oxford, UK, using X-radiation from the Al Kα band. All XPS experiments were recorded using an analyzer energy of 100 eV for survey scans and 20 eV for detailed scans. The base pressure in the analysis chamber was maintained at not more than 1.0 × 10^{-6} mbar. Each derivatised carbon black sample studied was directly mounted on double sided carbon adhesive tape (SPI supplies, PA, USA) attached on a copper stub and then placed in the ultra-high vacuum analysis chamber of the spectrometer. Analysis of the resulting spectra was performed using XPS Peak 4.1. Note that a spectral shift in binding energy caused by the instrument was taken into account in order to get the accurate binding energy for each element characterised. The reported atomic composition values for each sample were corrected with the appropriate atomic sensitivity factors.

UV-Visible spectroscopy was performed using a Varian Cary-100 Bio UV-Vis Spectrophotometer (Varian, Oxford, UK) with Cary WinUV software. Fluorescence spectroscopy was performed on a Varian Cary Eclipse Fluorescence Spectrophotometer (Varian, Oxford, UK) with Cary Eclipse software. The slit width for both excitation and emission monochromators was set to be 5 nm. Microscopy was performed using a custom built TIRF microscope (base: Eclipse TE2000-U, Nikon, UK). Samples were illuminated with a 473 nm laser (SDL-473-100T, Shanghai Dream, China) at a power of *ca.* 2 μW μm^{-1}, and the resulting fluorescence was imaged with an exposure of 20 ms on an EMCCD camera (DV860PCS-BV, iXon, Andor, US).

Electrochemical measurements were recorded using an Autolab PGSTAT 20 computer-controlled potentiostat (Eco-Chemie, Utrecht, The Netherlands) with a standard three-electrode configuration. A glassy carbon electrode (GC, 3 mm

diameter, BAS Technical, UK) was used as the working electrode. A saturated calomel electrode (SCE) and a carbon rod acted as the reference and counter electrodes respectively. The GC was polished using diamond pastes of decreasing sizes (Kemet, UK). Cyclic voltammetry was recorded at a scan rate of 50 mV s^{-1}. All solutions were thoroughly degassed with pure N_2 for 10 minutes prior to performing any voltammetric measurements. Electrochemical experiments were carried out at room temperature in 0.1 M sodium hydroxide solution.

3. Results and discussion

3.1 Synthesis of Fluorescein/CB

Carbon black was modified with fluorescein by physisorption of fluorescein on the CB surface. Simply, *ca.* 50 mg of CB was stirred into 50 mL of 10 mM NaOH solution containing 2.5 mM fluorescein for 48 h, isolated by centrifugation, washed extensively with 10 mM NaOH and deionised water to remove non-associated fluorescein and dried under vacuum to yield a physisorbed fluorescein-modified carbon black, labelled Fluorescein/CB.

3.2 Characterisation of Fluorescein/CB

The Fluorescein/CB sample was characterised by means of X-ray photoelectron spectroscopy and cyclic voltammetry.

Fig. 2 displays the X-ray photoelectron spectra over the range of 0–1400 eV for (a) native CB and (b) Fluorescein/CB. In both cases, two main peaks were clearly observed at *ca.* 285 and 532 eV corresponding to the emission from the C_{1s}, and O_{1s} levels, respectively.[29] Another two spectral peaks were observed at *ca.* 974 and 1224 eV corresponding to the O_{KLL} and C_{KLL} Auger emissions, respectively.[29] Detailed scans over the region of interest were recorded in order to obtain the quantitative data (Inset in Fig. 1). The percentage elemental composition of each element was determined from the area under each peak, and is shown in Table 1.

The results clearly demonstrate that after stirring CB in fluorescein solution for 48 h, the percentage of O increases to 4.8%, compared to 1.6% in the native CB, confirming the physisorption of fluorescein on CB surface. It is important to note that XPS characterisation of either Fluorescein/CB or native CB was performed under high vacuum condition ($\leq 10^{-6}$ mbar), therefore the CB surface was less likely to be contaminated by atmospheric oxygen or moisture, leading to the conclusion that the increase in the oxygen content observed at Fluorescein/CB sample corresponded to oxygen in the fluorescein molecule.

Next, the Fluorescein/CB was characterised by cyclic voltammetry in 0.1 M sodium hydroxide pH 12.80 solution. According to the literature, the reduction of fluorescein has been previously studied in 0.1 M NaOH (pH above 12) where a reversible one-electron reduction to a semireduced-fluorescein radical was observed, and shown in eqn (1).[30–32]

Fig. 3 displays the overlaid cyclic voltammograms of either (a) Fluorescein/CB or (b) native CB, drop-cast on a GC electrode and (c) a bare GC electrode, in 0.1 M NaOH. It can be seen from Fig. 3 that no reduction feature was observed for the unmodified CB (Fig. 3b) nor the bare GC electrode (Fig. 3c), whereas for the Fluorescein/CB (Fig. 3a), a reduction peak was observed at *ca.* −1.28 V *vs.* SCE which corresponds to the reduction of fluorescein, confirming the presence of fluorescein on the CB surface. However, on the reverse scan, no clear reverse oxidation peak for fluorescein was observed, possibly suggesting a reduction process followed by a chemisorption of the generated semireduced-fluorescein radical onto CB surface.[33–35] Previously, it has been reported in the literature that the aryl radical generated at the carbon surface appears to couple to the carbon surface *via* a formation of a covalent bond.[33–35]

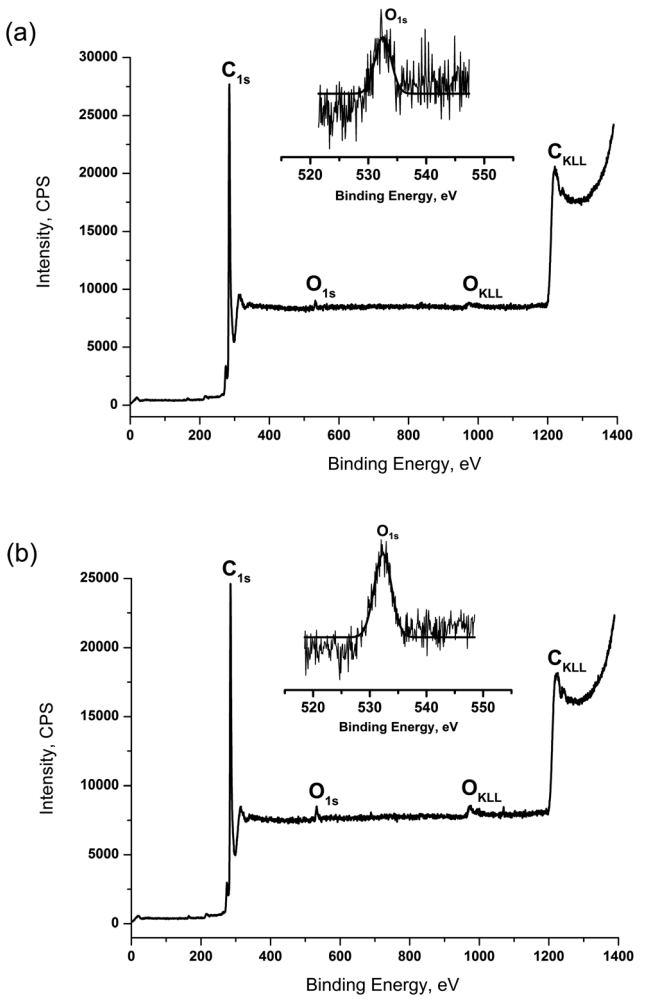
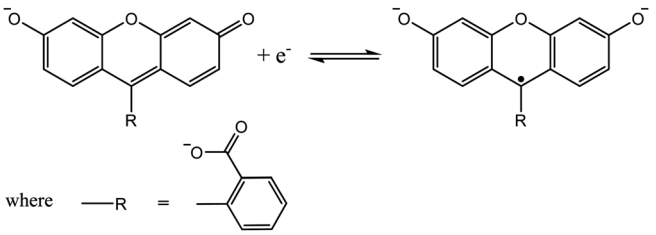


Fig. 2 Wide scan XPS spectra over the range of 0–1400 eV for (a) native CB and (b) Fluorescein/CB. Inset: detailed scans over O_{1s} region.

Table 1 The percentage element composition of (1) native CB and (2) Fluorescein/CB samples using XPS

Carbon black samples	%C	%O
Native CB	98.4	1.6
Fluorescein/CB	95.2	4.8



(1)

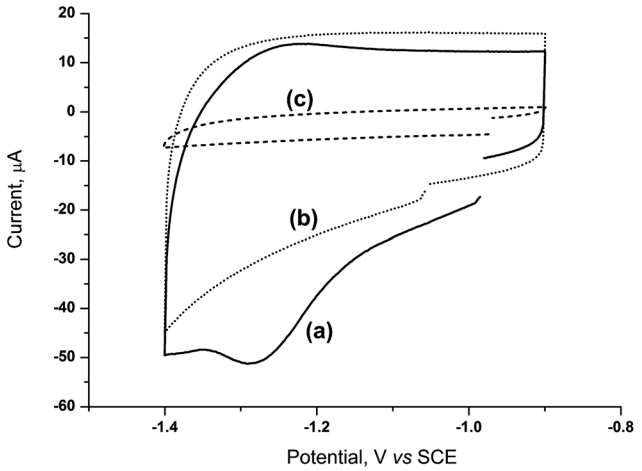


Fig. 3 Overlaid cyclic voltammograms of either (a) Fluorescein/CB or (b) native CB, drop-cast on a GC electrode, and (c) a bare GC electrode, in 0.1 M NaOH. All scan at 50 mV s^{-1}.

The approximate surface coverage, Γ (mol cm^{-2}), of fluorescein physisorbed on the CB surface can be estimated from the area under the reduction peak of fluorescein shown in Fig. 3a and then using eqn (2):[26]

$$\Gamma = \frac{Q}{nFA} \quad (2)$$

where Q is the charge passed (C), F is Faraday's constant (96 485 C mol^{-1}), A is the surface area of the Fluorescein/CB, drop-cast onto the GC electrode (determined from the diameter of CB together with the density of CB, and knowing the amount of CB drop-cast onto the electrode surface, cm^2), and n is the number of electrons transferred ($n = 1$). The surface coverage for fluorescein physisorbed on CB was therefore found to be 2.6×10^{-11} mol cm^{-2}.

3.3 Determination of fluorescence excitation and emission profiles of fluorescein in water

According to the literature,[18–21] fluorescein in different aqueous solutions with different pH values can exist in four different forms: cation, neutral, monoanion, and dianion (shown in Fig. 1), leading to different excitation and emission wavelengths of fluorescence. Therefore, the absorption and fluorescence emission of fluorescein in pure water was initially explored by UV-Vis and fluorescence spectroscopy in order to get the certain excitation (λ_{EX}) and emission (λ_{EM}) wavelengths for the Fluorescein/CB which was next investigated in deionised water. Note that at the pH value of water, fluorescein is primarily present in its dianionic form.[18–21]

Fig. 4 shows the UV-Vis absorption spectrum of 10 μM fluorescein in water. The wavelength of maximum absorbance (λ_{max}) was observed at *ca.* 485 nm. This wavelength was then employed as an excitation wavelength (λ_{EX}) for the fluorescence emission of 5 μM fluorescein in water, and its fluorescence emission spectrum (shown as an inset in Fig. 4) was observed at longer wavelength with the maximum fluorescence intensity (λ_{EM}) at 511.94 nm. This shows that once excited by light at wavelength of 485 nm, fluorescein in water medium emits fluorescence light at wavelength of 511.94 nm. Therefore, the excitation and emission wavelengths of 485 and 511.94 nm, respectively, were selected as operating wavelengths for the fluorescence emission of Fluorescein/CB.

3.4 Effect of oxygen on the fluorescence intensity of Fluorescein/CB

As highlighted in the literature,[16,36–39] molecular oxygen is a common fluorescence quencher for various fluorophores due to its high solubility in aqueous solutions and organic solvents. In particular, the fluorescence quenching of fluorescein by molecular oxygen in aqueous solution has previously been reported.[16] The results showed that fluorescence intensity of fluorescein decreased with increasing oxygen concentration, and a fluorescence quenching mechanism of fluorescein with molecular oxygen in the solution was also proposed.[16]

Since oxygen is a particularly good quencher which can quench almost all known fluorophores, it is often necessary to remove oxygen from the solution before measuring fluorescence spectra.[40] In this study, Fluorescein/CB suspended in water was bubbled with N_2 gas for a range of times in order to get rid of dissolved oxygen in the solution.[41]

Fig. 5 displays the fluorescence emission spectra of Fluorescein/CB, suspended in water in the concentration of 0.01 mg mL^{-1} with N_2 bubbling for 0 to 70 min at controlled N_2 flow rate

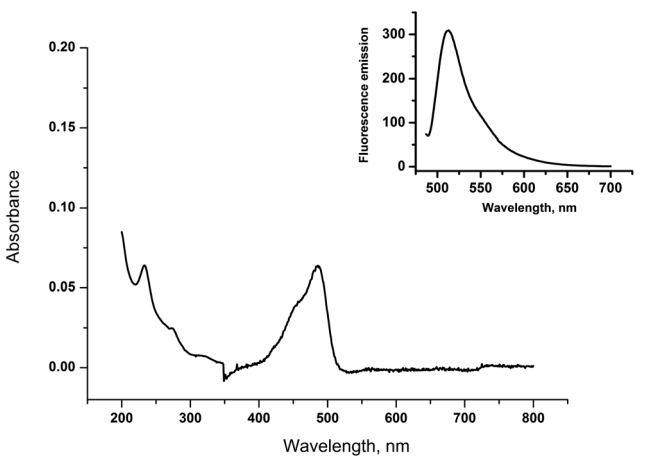


Fig. 4 UV-Vis absorption spectrum of 10 μM fluorescein in water medium, with maximum absorbance observed at *ca.* 485 nm. Inset: fluorescence emission spectrum of 5 μM fluorescein in water medium ($\lambda_{Excitation}$ at 485 nm) with emission maximum peak wavelength of 511.94 nm.

of 60 cm^3 min^{-1} through a 5 mL volume of the suspension. As seen in Fig. 5, the fluorescence intensity of Fluorescein/CB increased noticeably with increasing O_2 removal time. After 30 min of bubbling with N_2, the fluorescence intensity of Fluorescein/CB increased by *ca.* 14-fold compared to that without N_2 bubbling, and after this time the fluorescence intensity levelled off (plotted as an inset in Fig. 5), demonstrating that oxygen was almost entirely eliminated from the Fluorescein/CB suspension and a reliable fluorescence intensity measurement of Fluorescein/CB could then be obtained. According to these results, N_2 bubbling for 40 min at the controlled flow rate of 60 cm^3 min^{-1} through a volume of 5 mL was therefore chosen as the optimum experimental condition of O_2 removal for further studies.

3.5 Effect of Fluorescein/CB concentration on the fluorescence intensity

The effect of varying the amount of Fluorescein/CB, suspended in water medium on the fluorescence intensity was next investigated. Four different concentrations of Fluorescein/CB suspended in water (0.001, 0.01, 0.05 and 0.1 mg per mL of water) were prepared from a 0.1 mg mL^{-1} Fluorescein/CB stock solution by dilution. Each Fluorescein/CB sample was bubbled with N_2 for 40 min at controlled N_2 flow rate of 60 cm^3 min^{-1}, and its fluorescence emission spectrum was then measured by fluorescence spectroscopy with excitation wavelength of 485 nm. Fig. 6 displays the fluorescence emission spectra of Fluorescein/CB suspended in water in four different concentrations: 0.001 mg mL^{-1} (black), 0.01 mg mL^{-1} (blue), 0.05 mg mL^{-1} (green) and 0.1 mg mL^{-1} (red). It can be clearly seen from Fig. 6 that Fluorescein/CB in the concentration of 0.01 mg mL^{-1} (blue) showed the highest intensity of fluorescence measured at 511.94 nm.

Similar experiments were performed using unmodified CB and its fluorescence emission spectra are also displayed as an inset in Fig. 6. No visible fluorescence emission peak could be observed for the native CB, confirming the fluorescence property of fluorescein physisorbed on CB surface. Furthermore, Fluorescein/CB was suspended in deionised water for extended periods of time (48 h), centrifuged and the supernatant analysed. No trace of fluorescence was detected in the latter, confirming that no fluorescein leached from the Fluorescein/CB surface.

As for the reproducibility of the fluorescence intensity of Fluorescein/CB, six individual fluorescence experiments were performed on the Fluorescein/CB in the concentration of 0.01 mg mL^{-1}, with 40 min bubbling with N_2 before the fluorescence measurements. From the fluorescence emission spectra, it was determined that the relative standard deviation (%RSD) of fluorescence intensity measured at 511.94 nm was 6.5%, indicating good reproducibility of Fluorescein/CB.

As a consequence, all following results reported were obtained from Fluorescein/CB suspended in water in the concentration of 0.01 mg mL^{-1} with N_2 bubbling for 40 min at the controlled flow rate of 60 cm^3 min^{-1} before the fluorescence emission spectra were measured at 511.94 nm, with the excitation wavelength of 485 nm.

3.6 Fluorescence quenching of Fluorescein/CB by Pd(II)

It has been previously reported that Fe(III) at a concentration of 5×10^{-4} M (and above) could completely quench the fluorescence of 20 ppm fluorescein in water, and the quenching effects of Fe(III) on fluorescein fluorescence has also been discussed.[42] However, no research concerning the fluorescence quenching of fluorescein by Pd(II) has previously been carried out.

In this study, a qualitative on-off experiment confirming the fluorescence quenching of Fluorescein/CB by either Pd(II) or Fe(III) was first investigated by fluorescence microscopy. Simply, 0.01 M of either Pd(II) or Fe(III) was added into Fluorescein/CB suspension containing 0.01 mg of Fluorescein/CB per mL of water. All the sample solutions were then mixed thoroughly, bubbled with N_2 gas for 40 min and deposited onto a glass slide

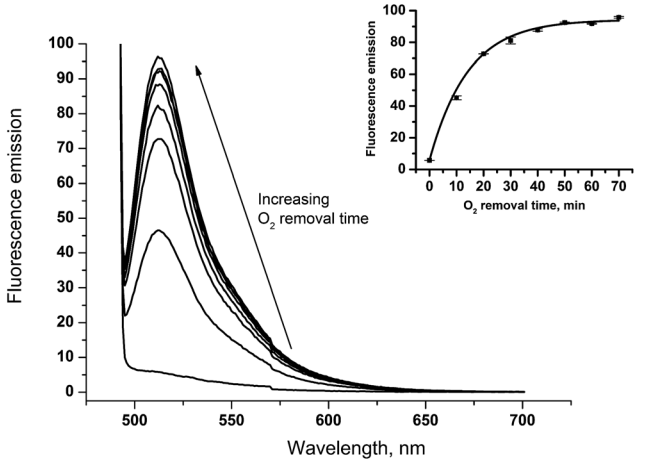


Fig. 5 Fluorescence emission spectra of Fluorescence/CB, suspended in water in the concentration of 0.01 mg mL^{-1} with N_2 bubbling for 0, 10, 20, 30, 40, 50, 60 and 70 min in order to remove O_2 from the sample solutions. Inset: plot of fluorescence intensity of Fluorescein/CB *versus* O_2 removal time.

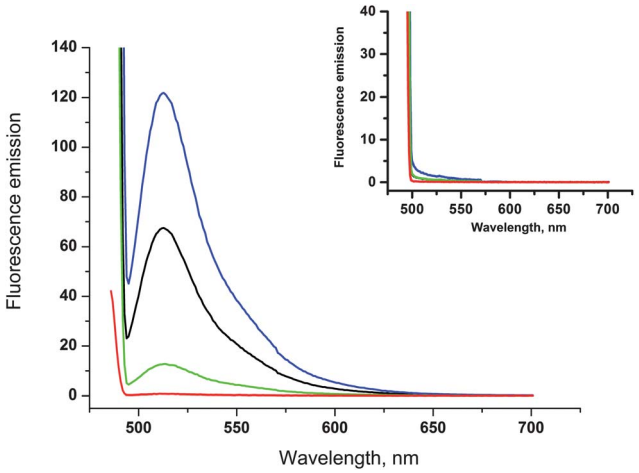


Fig. 6 Fluorescence emission spectra of Fluorescein/CB, suspended in water in the concentration of 0.001 mg mL^{-1} (black), 0.01 mg mL^{-1} (blue), 0.05 mg mL^{-1} (green) and 0.1 mg mL^{-1} (red). Inset: fluorescence emission spectra of unmodified CB, suspended in water in the concentration of 0.001 mg mL^{-1} (black), 0.01 mg mL^{-1} (blue), 0.05 mg mL^{-1} (green) and 0.1 mg mL^{-1} (red). All solutions were bubbled with N_2 gas for 40 min before fluorescence measurements.

for imaging. Fig. 7 displays the fluorescence images of Fluorescein/CB in the absence (a) and presence of either (b) 0.01 M Pd(II) or (c) 0.01 M Fe(III). As seen from the fluorescence images, Fluorescein/CB itself was very bright relative to the dark background, and the mean pixel count of this image was found to be saturating the camera at 16353.54, demonstrating the high intensity of fluorescence, whereas low imaging contrast relative to the dark background indicating a weak fluorescence signal with that the mean pixel count values of 5515.18 and 1864.04 could be observed for Fluorescein/CB in the presence of Fe(III) and Pd(II), respectively. These results clearly show that both Pd(II) and Fe(III) could quench the fluorescence of Fluorescein/CB with Pd(II) being more efficient in quenching. Blanks confirmed that the fluorescence emission was *not* observed for either Pd(II) or Fe(III) solutions.

Next, the fluorescence quenching of Fluorescein/CB by Pd(II) was examined quantitatively by fluorescence spectroscopy. Fig. 8 displays the fluorescence emission spectra of Fluorescein/CB, 0.01 mg mL^{-1} in water with varying concentration of Pd(II) from 0 to 10 μM. It can be seen from Fig. 8 that the fluorescence intensity of Fluorescein/CB decreased regularly but not linearly (Inset in Fig. 8) with the increase of Pd(II) concentration, while there was no change in emission maxima and shape of peaks during the quenching process, suggesting no observable photochemical reaction between Fluorescein/CB and Pd(II).[43]

Typically, fluorescence quenching process is investigated in two categories: dynamic (collisional) and static (complex formation) quenching.[16,17,40] The dynamic quenching mechanism results from diffusive collisions between the fluorophore and quencher during the lifetime of the excited state, whereas the static quenching occurs as a result of the formation of a non-fluorescent ground state complex between the ground-state fluorophore and quencher.[40] Additionally, the dynamic fluorescence quenching is generally described by the Stern–Volmer equation, shown in eqn (3).[16,17,40]

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (3)$$

where F_0 and F are the fluorescence intensities of the fluorophore in the absence and presence of the quencher, respectively. [Q] is the concentration of the quencher, and K_{SV} is the Stern–Volmer quenching constant. Quenching data are frequently presented as a plot of F_0/F *versus* [Q], as F_0/F is expected to be linearly dependent upon the concentration of quencher with an intercept of one on the y axis and a slope equal to K_{SV}.[16,17,40]

Fig. 9 displays the Stern–Volmer plot obtained for the fluorescence quenching of Fluorescein/CB with increasing Pd(II) concentration. As clearly seen from Fig. 9, the Stern–Volmer plot showed a good linear response over the Pd(II) concentration range of 0 to 3 μM, indicating that the quenching mechanism follows the collisional quenching process.[17,39,40] As the concentration of Pd(II) increased (≥5 μM Pd(II)), the Stern–Volmer plot exhibited a positive deviation from linearity (plotted as an inset in Fig. 9), possibly suggesting the simultaneous presence of both dynamic and static mechanism.[17,39,40] Similar experimental results, demonstrating that the fluorescence quenching is not purely dynamic, were also previously observed by others.[17,39,44,45] The Stern–Volmer quenching constant (K_{SV}) calculated from the data in Fig. 9 was found to be 5.2×10^5 mol^{-1} dm^3 (shown in Table 2). Detection and quantification limits[46] for the fluorescence quenching of Fluorescein/CB by Pd(II) in water medium are also included in Table 2, showing that the Fluorescein/CB is feasible and sensitive for the determination of trace amounts of Pd(II) in aqueous solution. It is important to note that the

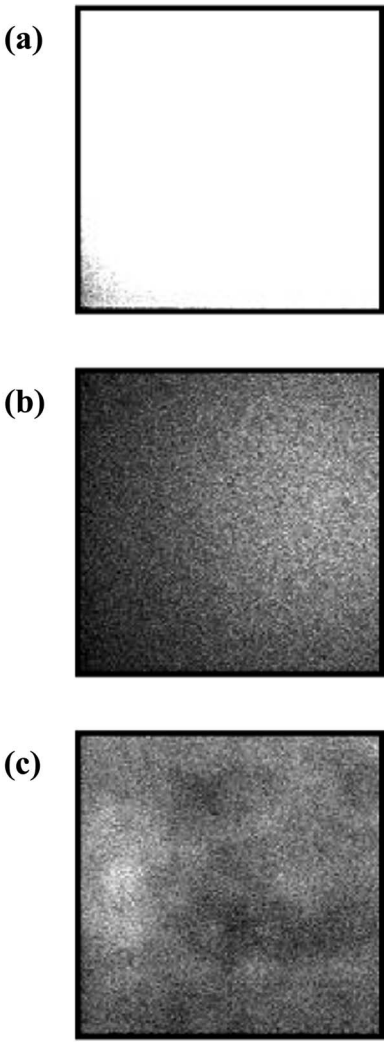


Fig. 7 Fluorescence images of 0.01 mg mL^{-1} Fluorescein/CB suspended in water, in the absence (a, mean pixel count: 16353.54) and presence of either 0.01 M Pd(II) (b, mean pixel count: 1864.04) or 0.01 M Fe(III) (c, mean pixel count: 5515.18). All solutions were bubbled with N_2 gas for 40 min before fluorescence measurements.

Table 2 Stern–Volmer quenching constant (K_{SV}),[40] detection and quantification limits[46] for the fluorescence quenching of Fluorescein/CB in water by Pd(II)

Metal ions	K_{SV}, 10^3 mol^{-1} dm^3	DL[a], 10^{-6} mol dm^{-3}	QL[b], 10^{-6} mol dm^{-3}
Pd(II)	517	1.07	3.57

[a] Detection limit (DL) defined as three times the standard deviation of the blank for $n = 10$. [b] Quantification limit (QL) defined as ten times the standard deviation of the blank for $n = 10$.

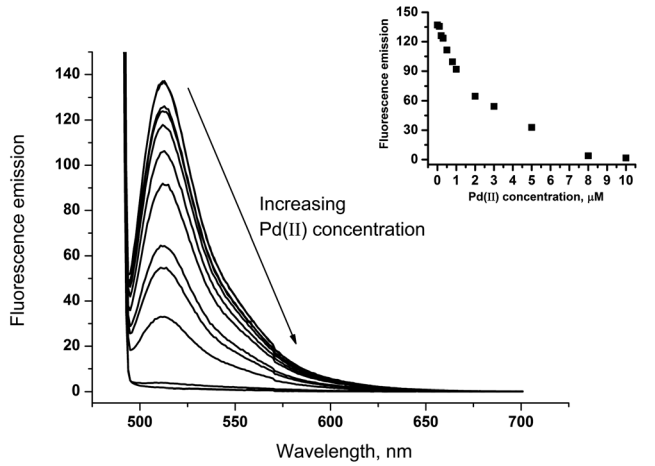


Fig. 8 Fluorescence emission spectra of Fluorescein/CB 0.01 mg mL^{-1} suspended in water, in the presence of Pd(II) in the concentration range of 0, 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, 3.0, 5.0, 8.0 and 10.0 μM. All solutions were bubbled with N_2 gas for 40 min before fluorescence measurements. Inset: plot of fluorescence intensity *versus* Pd(II) concentration present in the Fluorescein/CB suspension.

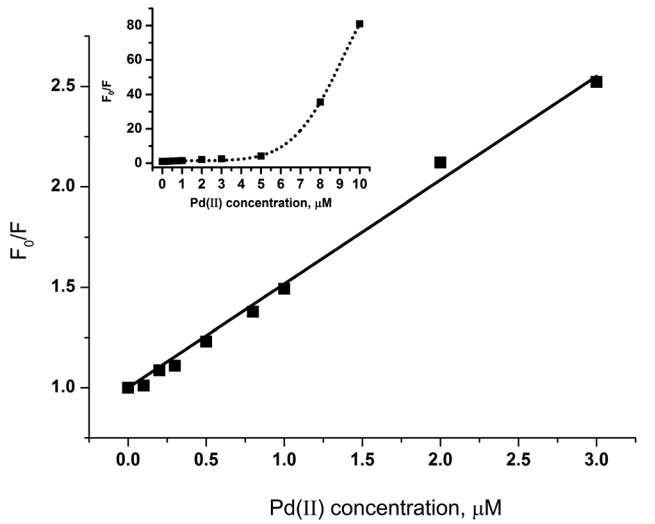


Fig. 9 Stern–Volmer plot for quenching of fluorescence of Fluorescein/CB 0.01 mg mL^{-1} suspended in water in the presence of Pd(II) in the concentration range of 0–3 μM, with correlation coefficient (R^2) of 0.998. Inset: Stern–Volmer plot for quenching of fluorescence of Fluorescein/CB 0.01 mg mL^{-1} suspended in water in the presence of Pd(II) in the concentration range of 0–10 μM.

detection limit (LOD) of 1.07 μM reported here is more than an order of magnitude below the concentration limit for Pd(II) suggested by the European Agency for the Evaluation of Medicinal Products (EMEA).[4,8]

3.7 Removal of Fe(III) from the system by formation of an Fe(III)–salicylic acid complex

Regarding the fluorescence quenching of Fluorescein/CB investigated earlier, the Fluorescein/CB was found to be responsive towards both Pd(II) and Fe(III). This means that Fe(III) interfering in the solution containing Pd(II) could produce a false positive response in the determination of Pd(II) by this fluorescence quenching of Fluorescence/CB method. Therefore, attempts were made to remove Fe(III) interference from the system by forming a complex with salicylic acid,[47] so that the Fluorescein/CB could be able to detect Pd(II) without being affected by Fe(III).

According to our previous work,[48] when salicylic acid was added to an Fe(III) solution, the colour of the solution changed from pale yellow to violet as a result of the formation of intensely coloured complexes between salicylic acid and Fe(III). In addition, it has been reported in the literature that the reaction between Fe(III) and salicylic acid results in the formation of a 1 : 1 and/or 1 : 2 Fe(III)–salicylic acid stable complex, as shown in eqn (4) and (5).[47–49] On the other hand, no experimental evidence of complexation between Pd(II) and salicylic acid has been reported.[47]

$$Fe^{3+} + C_7H_6O_3 \rightarrow [FeC_7H_4O_3]^+ + 2H^+ \quad (4)$$

$$Fe^{3+} + 2(C_7H_6O_3) \rightarrow [Fe(C_7H_5O_3)_2]^+ + 2H^+ \quad (5)$$

From these observations, it is expected that Fe(III) forms a stable complex with salicylic acid, resulting in the removal of free Fe(III) participating in the fluorescence quenching of Fluorescein/CB from the system, whereas Fluorescein/CB and Pd(II) stay unaffected in the presence of salicylic acid.

Fig. 10 displays the fluorescence emission spectra of 10 μM salicylic acid (yellow dashed line) and Fluorescein/CB suspension, in the absence (black dashed line) and presence (red dashed line) of 10 μM Fe(III), as well as the spectra of the same Fluorescein/CB suspension containing 10 μM Fe(III) after salicylic acid in the concentration of 10 μM (blue solid line), 20 μM (green solid line) and 30 μM (pink solid line) was added in order to remove free Fe(III) from the system by forming the Fe(III)–salicylic acid complex. The results clearly demonstrated that the fluorescence intensity of Fluorescein/CB decreased when 10 μM Fe(III) was present in the suspension. After an equimolar concentration (10 μM) of salicylic acid was added to the Fluorescein/CB suspension containing 10 μM Fe(III), the fluorescence intensity of Fluorescein/CB moderately increased, but still lower than that of Fluorescein/CB alone, suggesting the partial removal of Fe(III) by complexation with salicylic acid. The complete removal of Fe(III) from the system was achieved when excess amounts (20 and 30 μM) of salicylic acid were added to the 10 μM Fe(III)–Fluorescein/CB suspension as the fluorescence intensity of Fluorescein/CB recovered to its initial value obtained when no quenching occurred.

Similar experiments on complexation with salicylic acid were also carried out in the Fluorescein/CB suspension containing 10 μM Pd(II), and the resulting fluorescence emission spectra are displayed as an inset in Fig. 10. It can be clearly seen that the fluorescence intensity of Fluorescein/CB, initially quenched by 10 μM Pd(II) remained relatively unchanged after 10 and 20 μM salicylic acid were added into the 10 μM Pd(II)-Fluorescein/CB suspension, suggesting no complex formation between Pd(II) and salicylic acid.

In addition, further fluorescence quenching experiments were performed in the Fluorescein/CB suspension where both Pd(II)

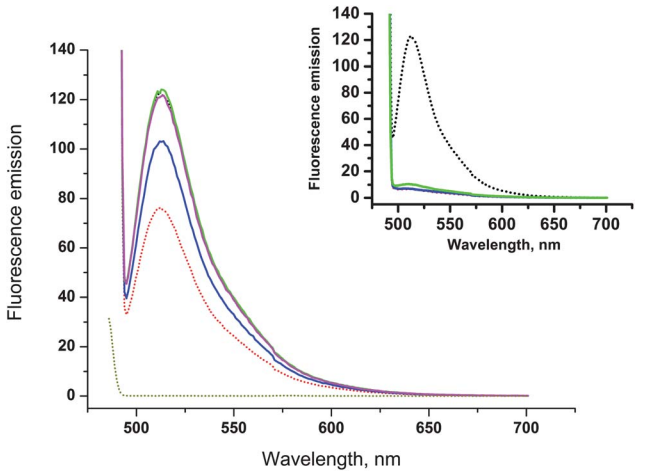


Fig. 10 Fluorescence emission spectra of 10 μM salicylic acid (yellow dashed line) and Fluorescein/CB suspension, in the absence (black dashed line) and presence (red dashed line) of 10 μM Fe(III). Also shown is the fluorescence emission spectra of the same Fluorescein/CB suspension in the presence of 10 μM Fe(III) after salicylic acid in the concentration of 10 μM (blue solid line), 20 μM (green solid line) and 30 μM (pink solid line) was added. Inset: fluorescence emission spectra of Fluorescein/CB suspension, before (black dashed line) and after (red dashed line) 10 μM Pd(II) was added, and then followed by an addition of salicylic acid in the concentration of 10 μM (blue solid line) and 20 μM (green solid line).

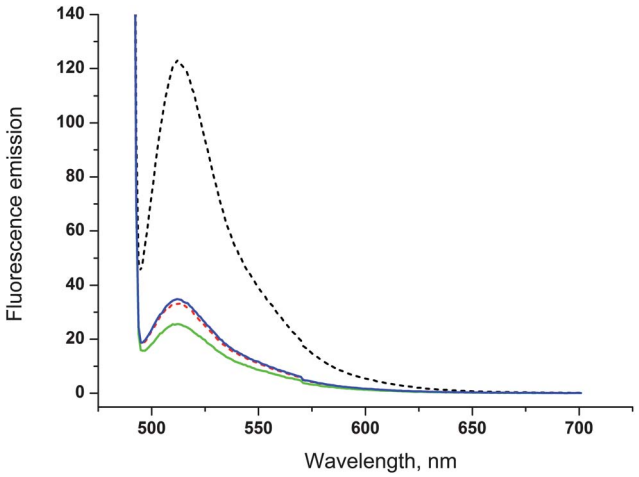


Fig. 11 Fluorescence emission spectra of Fluorescein/CB suspension, in the absence (black dashed line) and presence of 5 μM Pd(II), before (red dashed line) and after (green solid line) 5 μM Fe(III) was consecutively added. Also shown is the fluorescence emission spectrum of Fluorescein/CB suspension containing 5 μM Pd(II) and 5 μM Fe(III) after 10 μM salicylic acid was added (blue solid line).

and Fe(III) were present together. Fig. 11 displays the fluorescence emission spectra of Fluorescein/CB suspension, in the absence (black dashed line) and presence of 5 μM Pd(II), before (red dashed line) and after (green solid line) 5 μM Fe(III) was consecutively added, together with the spectra of the same Fluorescein/CB suspension containing 5 μM Pd(II) and 5 μM Fe(III) after 10 μM salicylic acid was added (blue solid line) in order to get rid of Fe(III) quenching the fluorescence intensity of Fluorescein/CB from the system. As seen in Fig. 11, the fluorescence intensity of Fluorescein/CB decreased as expected when 5 μM Pd(II) was present in the suspension, while further decrease in the fluorescence intensity was observed for the Fluorescein/CB suspension containing both 5 μM Pd(II) and 5 μM Fe(III). After Fe(III), present in the Fluorescein/CB suspension containing Pd(II), was removed by treatment with two equivalents of salicylic acid, the fluorescence emission intensity of Fluorescein/CB returned to the signal observed earlier in the Fluorescein/CB suspension containing only Pd(II), demostrating that this approach for removing Fe(III) could effectively control the selectivity of Fluorescein/CB towards Pd(II). It can therefore be concluded that Fluorescein/CB, a fluorescence-sensing material, is capable of highly selective detection of Pd(II) in aqueous solution in the presence of Fe(III).

4. Conclusions

A fluorescein-modified carbon black material (Fluorescein/CB) has been synthesised by an extremely facile method and characterised. The appropriate experimental conditions for the Fluorescein/CB were investigated in detail, and the Fluorescein/CB was then desirably used as a fluorescent sensor for the sensitive detection of Pd(II) in aqueous solution by fluorescence quenching. The results showed that the fluorescence intensity of Fluorescein/CB decreased constantly with the increase in the concentration of Pd(II) without any change in either emission maximum wavelength or peak shape, demonstrating that the interaction between Fluorescein/CB and Pd(II) existed without any photochemical reaction observed. The fluorescence quenching of Fluorescein/CB by Pd(II) could therefore described by the Stern–Volmer equation, and the Stern–Volmer plot showed a linear response over the Pd(II) concentration range of 0 to 3 μM, with a quenching constant (K_{SV}) of 5.17×10^5 mol^{-1} dm^3. Proportional response was observed between 0 and 10 μM Pd(II), and the limit of detection (LOD, defined as three time the standard deviation of the blank for $n = 10$) for Pd(II) was found to be 1.07 μM. In addition, attempted removal of Fe(III) interfering the fluorescence quenching of Fluorescein/CB was carried out by forming a complex with salicylic acid, and the results showed that Fe(III) could be effectively removed from the system without affecting Pd(II) in the solution. This suggested that Fluorescein/CB has the potential to be used as a sensitive and selective fluorescence sensor for Pd(II) in aqueous solution even in the presence of Fe(III).

Acknowledgements

Syngenta are acknowledged for both support and partial funding of this work. Cabot Corporation and James M Brown Ltd are acknowledged for the generous donation of carbon black used in this work, as well as supplying additional details regarding morphology, *etc.*

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