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Stable tetramethyl-1,10-phenanthroline osmium(III) complex in neutral pH as a photoluminescence-following electron-transfer reagent for the detection of acetaminophen in urine and pharmaceutical formulations†

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A stable $[Os(tmphen)_3]^{3+}$ (tmphen = 3,4,7,8-tetramethyl-1,10-phenanthroline) reagent was prepared in neutral aqueous solution by oxidation of $[Os(tmphen)_3]^{2+}$ with lead(IV) oxide. $[Os(tmphen)_3]^{2+}$ and $[Os(tmphen)_3]^{3+}$ is characterized by absorption spectroscopy. $[Os(tmphen)_3]^{3+}$ stability is compared with $[Ru(tmphen)_3]^{3+}$ in the same pH 7 environment. The properties of Os(III) and Ru(III) complex were investigated for use as the oxidant in a photoluminescence-following electron-transfer (PFET) system. Studies of photophysical and electrochemical properties, the stability of the Os(III) and Ru(III) complexes state in oxidizing environments, and analytical application in PFET detection of oxidizable pharmaceutical: acetaminophen (paracetamol) is presented. The limit of quantification (LOQ) was 30.2 μg L^{-1} and 1.5 μg L^{-1} was the limit of detection (LOD). 2x-1-Dimensional Solid Phase Extraction (2x-1D SPE) method was developed for determination of acetaminophen in urine. This method uses both the methanol concentration and the pH advantageously to preferentially isolate acetaminophen from complex sample matrix. Acetaminophen was detected in urine samples in a concentration range between 40.41 μg L^{-1} and 360.0 μg L^{-1}. Recoveries of greater than 90% were obtained with this selective method. The method was successfully applied to the determination of acetaminophen in commercial pharmaceutical formulations.

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1. Introduction

Hercules and Lytle were the first to observe a bright orange emission when an acidic solution of tris(2,2′-bipyridine)ruthenium(III) ($Ru(bpy)_3^{3+}$) reacted with concentrated sodium hydroxide or hydrazine.[1] Since that time, $[Ru(bpy)_3]^{2+}$ and its derivatives have emerged as versatile reagents in chemiluminescence (CL) and electrochemiluminescence (ECL) detection. Numerous analytical applications have included the selective determination of various analytes, which include oxalate, biomolecules (histamine and proline), alkaloid (sophoridine), pharmaceuticals (procyclidine, tramadol and lidocaine) and certain carboxylic acids(ethylenediaminetetraacetic acid and nitrilotriacetic acid), amines (triethylamine) and amino acids (valine and serine).[2–8] A comprehensive and critical review of the analytical applications of $[Ru(bpy)_3]^{3+}$ has been published recently.[9] Independent of the application, the chemistry is based on the chemical or electrochemical oxidation of stable $[Ru(bpy)_3]^{2+}$ to form $[Ru(bpy)_3]^{3+}$. There is a subsequent reaction with a suitable analyte (reducing agent) to generate $[Ru(bpy)_3]^{2+}$* in the triplet excited state, which returns to the ground state by emission of a photon. The emission response is quantitative.[2–8]

The major limitation of this redox cycle is the instability of the $[Ru(bpy)_3]^{3+}$ species in aqueous solutions. Studies have shown that solutions of $[Ru(bpy)_3]^{3+}$ deteriorate more rapidly at pH levels of 3.[10] Considering that the standard reduction potential for the $[Ru(bpy)_3]^{3+/2+}$ couple is 1.26 V, the instability of the reagent arises from its ability to oxidize water.[11] Recently, this problem has been solved by off-line generation of $[Ru(bpy)_3]^{3+}$ in acidic solutions. For instance, McDermott and co-workers established two methods for maintaining the stability of $[Ru(bpy)_3]^{3+}$ solutions; both used lead dioxide as the oxidant.[12] The first involved the off-line generation of $[Ru(bpy)_3]^{3+}$ in acetonitrile (containing 0.05 M $HClO_4$) and second in 95 : 5 glacial acetic acid-acetic anhydride (containing 0.05 M H_2SO_4).[12] These approaches have been successfully utilized for sensitive chemiluminescence detection of selected analytes, and since$[Ru(bpy)_3]^{3+}$ was generated in acidic non-

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aqueous environments the solution of the reagent was found to be stable over a 48 hour period.[12] Additional attempts have been made to generate stable $[Ru(bpy)_3]^{3+}$ solutions off-line by increasing the sulfuric acid concentration to 2.0 M.[13] This resulted in analytically useful chemiluminescence over 280 hour period for detection of codeine.[13] However this method had some disadvantages compared to non-aqueous systems: it required high buffer concentrations and there were significant variations in signal intensity. So the challenge remains in producing $[Ru(bpy)_3]^{3+}$ or its derivatives with long-term stability without compromising its merits of analytical performance.

The extensive use of $[Ru(bpy)_3]^{2+}$ in various detection systems has sparked a great interest in alternative metal-complexes to improve the sensitivity of existing detection systems and develop new analytical applications. Consider $[Ru(bpy)_3]^{2+}$ and $[Os(bpy)_3]^{2+}$. Both complexes have intense MLCT (metal-to-ligand charge transfer) bands and π–π^* transitions are nearly identical.[14] Despite the apparent similarities of these complexes, the excited-state properties are drastically different. These are best understood by referring to the positions of their lowest d–d, MLCT, and π–π^* triplet states.[15] The position of these states depends on the ligand field strength (Δ) of the heavy metal. Osmium complexes have larger Δ values than Ru(II) complexes given the same ligand field.[15] This raises the energy of the non-emissive d–d states, which reduces thermal deactivation of the MLCT states. As a result, Os(II) complexes have greater photostability compared to Ru(II) complexes. However, owing to differences in oxidation potentials and larger spin-orbit coupling, Os(II) complexes generally have lower emission energies and shorter excited-state lifetimes than Ru(II) analogues.[15] In more recent studies by Zammit *et al.*, these shortcomings have been addressed by substituting one or more polypyridial type ligands with stronger π-acceptor, such as diarsine or diphosphine, ligands. Similar to observations for Ru(III) complexes containing phenanthroline ligands, the oxidized analogues of Os(III) complexes containing diphosphine or diarsine ligands were found to be less stable in aqueous solution than $[Ru(bpy)_3]^{3+}$.[16] Despite the stability concerns, limit of detection for ofloxacin $[Os(phen)_2(dppene)]^{3+}$ chemiluminescence in acidic aqueous solution (0.05 M H_2SO_4) was 1.8 nM.[16]

Similarly, Jung *et al.* developed a photoluminescence-following electron-transfer (PFET) detection method for oxidizable analytes.[17] The detection scheme is based on reduction of oxidant by chromatographic eluent to generate a quantitative luminescence signal (Scheme 1). To date, PFET applications have used $[Ru(bpy)_3]^{3+/2+}$ and $[Os(bpy)_3]^{3+/2+}$ as oxidants for quantitative determination of peptides,[35] explosives,[18] and neurotransmitters.[36] $[Os(bpy)_3]^{3+}$ was found to be a more valuable PFET reagent compared to $[Ru(bpy)_3]^{3+}$ due to its fast electron-transfer exchange rate and greater stability in oxidation state (III).[19] However, similar to the $[Os(phen)_2(dppene)]^{3+}$ chemiluminescence detection system, acidic or non-aqueous environments were required to prevent greater than 10% reversion of $[Os(bpy)_3]^{3+}$ to $[Os(bpy)_3]^{2+}$.[17]

The work described here has two foci: (1) extension of the earlier work by Zammit *et al.* on Os(III) complexes containing strong π-acceptor ligands[16] and the work of Jung *et al.* on the use of $[Os(bpy)_3]^{3+}$ complex as a PFET reagent[17] and (2) assessment of a novel Os(III) complex as PFET reagent. The goals of this work are to overcome the challenge of producing an Os(III) complex with long-term stability in a neutral pure-aqueous environment and to assess its potential as PFET reagent. Herein we describe stable $[Os(tmphen)_3]^{3+}$ as a novel reagent in neutral aqueous solution for PFET detection in comparison to $[Ru(tmphen)_3]^{3+}$ (see Fig. 1 for full ligand names and structures). Tetramethyl-1,10-phenanthroline (tmphen) ligand was chosen because of the electron donating nature of the methyl substituents which can stabilize the metal complex in higher oxidation state. Photophysical and electrochemical properties, the stability of the Os or Ru(III) state using chemical oxidation, and its analytical application in detection of oxidizable pharmaceutical—acetaminophen (paracetamol) is presented. Numerous analytical methods have quantified acetaminophen

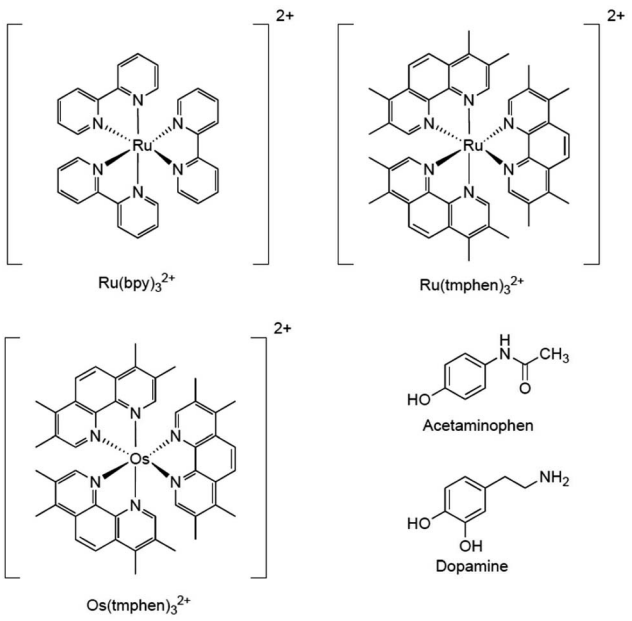
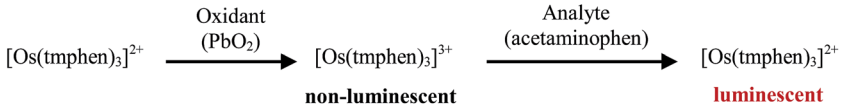


Fig. 1 Structures of tris(2,2′-bipyridine)ruthenium(II) $[Ru(bpy)_3^{2+}]$, tris(3,4,7,8-tetramenthyl-1,10-phenanthroline)ruthenium(II) $[Ru(tmphen)_3^{2+}]$, tris(3,4,7,8-tetramenthyl-1,10-phenanthroline)osmium(II) $[Os(tmphen)_3^{2+}]$, and acetaminophen.



Scheme 1 Generalized pathway for the production of Os(III) specie and subsequent regeneration of the emitting specie, $[Os(tmphen)_3]^{2+}$.

mainly for forensic purposes and to detect high concentrations of acetaminophen in human blood as occurring after overdosing.[31] Additional analytical methods are designed to quantify acetaminophen in rat plasma or urine for metabolism studies for *in vitro* or *in vivo* assays or for pharmaceutical quality controls.[32,33] Therefore, the foremost aim of the present work was to develop a robust and reliable method for the determination of acetaminophen in human urine including trace levels in the low $\mu g\ L^{-1}$ range.

2. Experimental

2.1 Reagents

Tris(2,2′-bipyridine)ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), potassium hexachloroosmate(IV) (K_2OsCl_6), 3,4,7,8-tetramethyl-1,10-phenanthroline (tmphen), ethylene glycol, acetaminophen and lead dioxide (PbO_2) were obtained from Aldrich (Milwaukee, WI). Phosphate buffer solution (PB, J. T. Baker, Phillipsburg, NJ, 0.10 M phosphate, 0.10 M sodium chloride, pH 7.40) was used in electrochemical experiments. PB solution (0.05 M, pH 7.0) was used in photoluminescence (PL) experiments. All aqueous solutions were prepared with deionized water (18.2 MΩ cm resistivity) from a Millipore Milli-Q Synthesis A10 system (Billercia, MA). SPE column (Oasis HLB, 2.1 mm × 20 mm; 30 μm) and Vacuum Manifold for SPE columns were purchased from Waters (Waters Corporation, MA, USA). All evaporations were performed with a 12-port N-Evap Model 111 evaporator (Organomation Associates Inc, Berlin, MA, USA). Stock solutions of buffers were prepared as follows: Na_2HPO_4–NaH_2PO_4 (0.1 M, pH 7.0), Na_2HPO_4–NaH_2PO_4 (0.1 M, pH 8.0), Na_2CO_3–$NaHCO_3$ (0.1 M, pH 9.2) and Na_2CO_3–$NaHCO_3$ (0.1 M, pH 10.8). The pH 7 phosphate-buffered saline (PBS) solution was prepared as follows: 7.2 g NaCl, 0.37 g KCl, 0.17 g $CaCl_2$, 13.8 g NaH_2PO_4 and 14.196 g Na_2HPO_4 were dissolved in a 1 L flask and adjusted to pH 7.0 to a final volume of 1 L.

2.2 Synthesis of $Os(tmphen)_3(Cl)_2$

The method was adapted from Zhang *et al.*[19] The salt K_2OsCl_6 (0.101 mmol) was dissolved in 10 mL of ethylene glycol, 3,4,7,8-tetramethyl-1,10-phenanthroline (0.32 mmol) was added in this solution. The solution was then refluxed for 3 hours and cooled to room temperature. The product was isolated by drop wise addition of the ethylene glycol solution into mixture of 10 mL acetone and 40 mL of ether. The procedure caused the precipitation of a solid, which was then filtered. The solid was dissolved in ethanol and the solution was filtered to remove potassium chloride. The product was further purified by dropwise addition of the ethanol solution into 100 mL of ether. This caused the precipitation of product, which was isolated by filtration and dried under vacuum. Zhang *et al.*[19] reported the synthesis, but NMR and photophysical properties of the complex were not reported. ^{1}H-NMR (Fig. S5†) of $Os(tmphen)_3(Cl)_2$: DMSO (δ): 2.21 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 7.58 (s, 1H) and 8.46 (s, 1H). ATR-FTIR of $Os(tmphen)_3(Cl)_2$ (Fig. S7†) shows frequencies that are similar to those reported of tmphen (3,4,7,8-tetramethyl-1,10-phenanthroline):[37] 2916 (C–H stretch of methyl), 1648, 1620 and 1566 (C=C, C=N stretches), 1446 and 1422 (C=C stretches), 1385 and 1285 (C–CH_3 stretch), 1197 (C–H bend), 1043 and 947 (CH_3 rock), 892 (C–H wagg.), 828 and 749 (Ring def.), 722 (Ring tors.) and 582 (Ring def.). ESI-MS data for $Os(tmphen)_3(Cl)_2$ and $Ru(tmphen)_3(Cl)_2$ were obtained using an Agilent 6520 Accurate-Mass Q-TOF LC/MS. ESI-MS (Fig. S9†): 450.1817 *m*/*z*, $[Os(tmphen)_3 + 2H]^{+2}$; 899.3511 *m*/*z*, $[Os(tmphen)_3 + H]^+$; and 935.3265 *m*/*z*, $[Os(tmphen)_3 + K]^+$. The purity of the compound is further determined by comparing the percentage of peak area from Extracted Ion Chromatogram (EIC) to that of the Total Ion Chromatogram (TIC). The $Os(tmphen)_3(Cl)_2$ show purity of 99.2%.

2.3 Synthesis of $Ru(tmphen)_3(Cl)_2$

The method was adapted from Kober *et al.*[20] The salt K_2RuCl_6 (0.101 mmol) was dissolved in 10 mL of ethylene glycol, 3,4,7,8-tetramethyl-1,10-phenanthroline (0.32 mmol) was added in this solution. The solution was then refluxed for 3 hours and cooled to room temperature. The product was isolated by dropwise addition of the ethylene glycol solution into a mixture of 10 mL acetone and 40 mL of ether. The resulting precipitate was then filtered. The solid was dissolved in ethanol and the solution was filtered to remove potassium chloride. The product was further purified by drop wise addition of the ethanol solution into 100 mL of ether. This caused the precipitation of product, which was isolated by filtration and dried under vacuum. Kober *et al.*[20] reported the synthesis, but NMR and photophysical properties of the complex were not reported. ^{1}H-NMR (Fig. S6†) of $Ru(tmphen)_3(Cl)_2$: DMSO (δ): 2.21 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 7.67 (s, 1H) and 8.47 (s, 1H). ATR-FTIR of $Ru(tmphen)_3(Cl)_2$ (Fig. S8†) shows frequencies that are similar to those reported of tmphen (3,4,7,8-tetramethyl-1,10-phenanthroline):[37] 2921 (C–H stretch of methyl), 1621, 1573 and 1512 (C=C, C=N stretches), 1447 and 1423 (C=C stretches), 1382 and 1263 (C–CH_3 stretch), 1185 (C–H bend), 1007 (Ring def.), 973 (CH_3 rock), 898 and 806 (C–H wagg.), 718 (Ring tors.), 656 and 578 (Ring def.). ESI-MS (Fig. S10†): 405.1514 *m*/*z*, $[Ru(tmphen)_3 + 2H]^{+2}$ and 845.2684 *m*/*z*, $[Os(tmphen)_3(Cl) + H]^+$. The purity of the compound is further determined by comparing the percentage of peak area from Extracted Ion Chromatogram (EIC) to that of the Total Ion Chromatogram (TIC). The $Ru(tmphen)_3(Cl)_2$ show purity of 99.0%.

2.4 Absorbance and stability Measurements of $[Ru(tmphen)_3]^{2+/3+}$ and $[Os(tmphen)_3]^{2+/3+}$

UV-Vis absorption spectra were collected using a Shimadzu UV-1800 spectrophotometer with 1 cm path length quartz cell. The relative stability of the $[Ru(tmphen)_3]^{3+}$ and $[Os(tmphen)_3]^{3+}$ state after oxidation with lead dioxide (PbO_2) was examined by adding 3 mg of PbO_2 to 3 mL of the $[Ru(tmphen)_3]^{2+}$ or $[Os(tmphen)_3]^{2+}$ complex (0.73 mM in DI water adjusted to required pH). The oxidized complex was injected through a Celltreat filter into a cuvette within the spectrophotometer, and the absorption peaks corresponding to the Ru or Os(II) and Ru or Os(III) states were monitored over time. Absorbances at 436

nm were compared to the absorbance of $[Os(tmphen)_3]^{2+}$ at 436 nm to calculate the amount of reversion from $[Os(tmphen)_3]^{3+}$ to $[Os(tmphen)_3]^{2+}$. Absorbances at 750 nm were compared to the absorbance of Ru(II) complex at 750 nm to calculate the amount of reversion from Ru(III) to Ru(II) complex.

2.5 Standard preparation and stock solutions

The acetaminophen stock solution was prepared by dissolving 14.8 mg acetaminophen in 0.1 M pH 7 buffer using a 10 mL plastic tube. For analysis, four calibration standards were prepared by gradual dilution with 0.1 M pH 7 buffer to final concentrations in a range from 74.10 μg L^{-1} to 40 000 μg L^{-1}. Stock solutions were stored at −20 °C in plastic tubes until further use.

2.6 Sample collection and preparation

Urine sample was collected with respect to ethical guidelines and permission of institutional review board and in compliance with the relevant laws and institutional guidelines. Urine samples were collected in 250 mL polyethylene containers and immediately stored at −20 °C. All samples were equilibrated to room temperature prior to analysis. Samples were vortex before transferring 400 μL aliquots into teflon vial. 200 μL of acetonitrile was added to each samples and were frozen at −20 °C over night to precipitate proteins. After thawing, all samples were vortex for two minutes and centrifuged at 3500 rpm for 10 min. The supernatant was transferred into second teflon vial.

A commercial pharmaceutical formulation, Tylenol™ tablets (containing 500 mg of acetaminophen), were analyzed. Determination of acetaminophen in the tablets was performed by weighing 5 tablets individually, then ground and mixed well. An 500 mg of tablet powder was accurately weighted, dissolved in DI water (50 mL) and sonicated for 20 minutes. The dissolved sample was filtered through Millipore filter paper (type ATTP, 0.8 μm). The filtrate was transferred to the 100 mL volumetric flask and diluted with DI water to the mark. Aliquots from 100 mL solution were further diluted with 0.1 M pH 7 buffer solution to obtain appropriate concentrations for analysis.

2.7 2x1D-solid phase extraction (SPE, wash-elute study)

2.7.1 1D-SPE: pH dependence. The wash-elute study is used to determine the pH needed in the wash step(s) and in the elution steps for the 2x1D-SPE of acetaminophen from urine matrix. Four Oasis HLB columns were mounted onto vacuum manifold for simultaneous processing. The columns were preconditioned with 2 mL of methanol under the vacuum (approx. 5 in. Hg, flow rate 1.0 mL min^{-1}). Subsequently, pressure was increased to 10 in. Hg and columns were allowed to run dry for 10 minutes (step 1). The vacuum was reduced to 5 in. Hg and columns were equilibrated with 2 mL of DI water (flow rate 1.0 mL min^{-1}). The Vac-Elut valve was closed as soon as the water reached the top of the sorbent bed to prevent columns from drying (step 2). During the remainder of the protocol, precaution was taken in-between steps to prevent columns from drying. An aliquot of 2 mL sample solution (15.18 μg mL^{-1} of acetaminophen in pH 7.0 phosphate buffer solution) was loaded onto each column, the Vac-Elut valve was opened and aliquot was drawn slowly trough the column (5 in. Hg, 1.0 mL min^{-1}) (step 3). The acetaminophen was eluted from each column with 2 mL 0.1 M buffer solution of pH 7.0, 8.0, 9.2 and 10.8, respectively (step 4). All eluates were collected separately and analyzed by UV-Vis spectrometer to determine the percent recovery of acetaminophen.

2.7.2 1D-SPE: methanol dependence. The wash-elute study is used to determine the percentage of methanol concentration needed in the wash step(s) and in the elution steps for the 2x1D-SPE of acetaminophen from urine matrix. Seven Oasis HLB columns were mounted onto Vacuum Manifold for simultaneous processing. The columns were preconditioned with 2 mL of methanol under the vacuum (approx. 5 in. Hg, flow rate 1.0 mL min^{-1}). Subsequently, pressure was increased to 10 in. Hg and columns were allowed to run dry for 10 minutes (step 1). The vacuum was reduced to 5 in. Hg and columns were equilibrated with 2 mL of DI water (flow rate 1.0 mL min^{-1}). The Vac-Elut valve was closed as soon as the water reached the top of the sorbent bed to prevent columns from drying (step 2). During the remainder of the protocol, precaution was taken in-between steps to prevent columns from drying. An aliquot of 2 mL sample solution (15.18 μg mL^{-1} of acetaminophen in pH 7.0 phosphate buffer solution) was loaded onto each column, the Vac-Elut valve was opened and aliquot was drawn slowly trough the column (5 in. Hg, 1.0 mL min^{-1}) (step 3). Each column was washed with 6 mL of DI water (step 4). The acetaminophen was eluted from each column with methanol–water mixtures of increasing methanol concentration (0, 0.25, 0.5, 1, 5, 10 and 30% methanol), respectively. All eluates were collected separately and analyzed by UV-Vis spectrometer to determine the percent recovery of acetaminophen.

2.8 2x1D-solid phase extraction: urine analysis

Non-spiked and spiked human urine samples were analyzed with acetaminophen to cover the desired concentration range. Three levels of acetaminophen concentrations were chosen (0.041, 0.12 and 0.36 μg mL^{-1}) to reflect the broad spectrum of urinary matrix. These spiked urine samples were prepared in pH 7.0 phosphate buffer solution (400 μL of urine with final volume of 2 mL). The sample was loaded onto the column, which had been preconditioned and equilibrated by following the previous 1D-SPE protocols. After loading the sample, 2x1D-SPE method was carried out as follows.

For the first 1D-SPE method, after loading the sample, column was washed with 2 mL of pH 7.0 buffer, 2 mL of pH 8.0 buffer, 2 mL of pH 9.2 buffer and then acetaminophen was eluted with 2 mL of pH 10.8 buffer.

For the second 1D-SPE method, pH of the eluate from the previous column was readjusted to 7.0 with concentrated hydrochloric acid and then loaded on the column. Subsequently, column was washed with 2 mL DI water and then acetaminophen was eluted with 2 mL of 0.25% methanol–water mixture. Overall 2x1D-SPE protocol is shown in Fig. S1.†

Precaution was taken in-between steps to prevent column from drying. All the above eluates were evaporated to dryness at

60 °C under a gentle stream of nitrogen and reconstituted with 2 mL of 0.1 M pH 7.0 buffer solution. The acetaminophen concentrations were measured by following PFET detection protocol in Section 2.11. The luminescence signal of the native samples were subtracted from the spiked samples to calculate percent recovery of acetaminophen.

2.9 Photoluminescence measurements

The concentrations of the samples for photoluminescence experiments were determined in water by UV/Vis absorption spectroscopy. The extinction coefficients used for $[Os(tmphen)_3]^{2+}$ and $[Ru(tmphen)_3]^{2+}$ complexes are given under "spectroscopic properties" in the Results section.

The luminescence of both the complexes was measured using a Photon Technology Instruments (PTI) fluorimeter at 20 °C, with a fixed concentration of 8.78 μM for both of the complexes. A septum-topped thermostated quartz cuvette was used. The cuvette had internal dimensions of 1.0 × 1.0 cm. All samples were equilibrated at 20 °C for one min before each measurement. Excitation was performed at 550 nm and emission was collected in the wavelength range of 600–900 nm. The excitation and emission slits were 2.5 and 20 nm, respectively. Scans were taken with a 3 nm step size using an integration time of 1.0 s point^{-1}. The luminescence from an average of three consecutive scans were used for analyses.

2.10 Cyclic voltammetry

CV measurements were performed using a model CH660B potentiostat (CH instruments, Inc., Texas). A three-electrode assembly was used consisting of a 3.0 mm diameter glassy carbon working electrode, a platinum wire auxiliary electrode and 5 cm^3 cell. A Ag/AgCl (sat. KCl) reference electrode was used. Samples consisted of $Os(tmphen)_3(Cl)_2$ (0.40 mM) or $Ru(tmphen)_3(Cl)_2$ (0.40 mM) in 0.10 M citric acid/phosphate (Na_2HPO_4) buffer at pH of 3, 5 and 7.4 with 0.10 M NaCl. The scans\rate was 0.2 V s^{-1} over a potential range of 0–0.8 V. A fresh solution was used for each measurement and all the electrodes were cleaned with DI water in between measurements. Data were collected and analyzed using the CH Instruments software package.

2.11 Photoluminescence-following electron-transfer (PFET) detection

Analytical application of $Os(tmphen)_3(Cl)_2$ as a PFET reagent was evaluated using a Photon Technology Instrument (PTI) fluorimeter. The relative PFET intensities for the reactions between $[Os(tmphen)_3]^{3+}$ and analyte (acetaminophen) were measured as following: 1.0 mM stock solution of $[Os(tmphen)_3]^{2+}$ was prepared in 0.10 M phosphate buffer solution (pH 7.0). A 2 mL solution of 17.56 μM $[Os(tmphen)_3]^{2+}$ in 0.10 M phosphate buffer solution, pH 7.0, was prepared from 1.0 mM stock solution. A 17.56 μM solution was oxidized off-line with lead dioxide, the oxidant was filtered off and 1000 μL of resulting $[Os(tmphen)_3]^{3+}$ solution was placed in a quartz cuvette. A 1000 μL solution of analyte (acetaminophen) in 0.10 M phosphate buffer solution (pH 7.0) was injected into a 1000 μL of Os(III) solution. The final concentration of the complex and phosphate buffer was, thus, 8.78 μM and 0.10 M, respectively. The analyte concentration was varied to establish its limit of detection and linear dynamic range. The relative PFET intensity of the final solution was measured using identical experimental parameters as photoluminescence measurements of the osmium complex.

Similar protocol was applied to analyze urine and pharmaceutical samples, with the exception of analyte solution. A 1000 μL of the reconstituted eluant from Section 2.8 was used for urine analysis. After mixing the analyte solution with Os(III) solution, the final concentration of acetaminophen were 0.041, 0.12 and 0.36 μg mL^{-1}.

3. Results and discussion

3.1 Photophysical properties of complexes

The following extinction coefficients were used: $[Ru(bpy)_3]^{2+}$, $\varepsilon_{452} = 14\,700$ M^{-1} cm^{-1} (ref. 21) and $[Os(tmphen)_3]^{2+}$. $\varepsilon_{609} = 7483$ M^{-1} cm^{-1} was experimentally determined in water (pH 7). The absorption spectrum of $[Os(tmphen)_3]^{2+}$ is shown in Fig. 2.

The absorption spectra of $[Os(tmphen)_3]^{2+}$ and $[Ru(tmphen)_3]^{2+}$ complex are similar to that of Ru(II)- and Os(II)-based polypyridine complexes and can be interpreted accordingly.[22] The high-intensity absorption band ($\lambda = 265$ nm) in the UV region

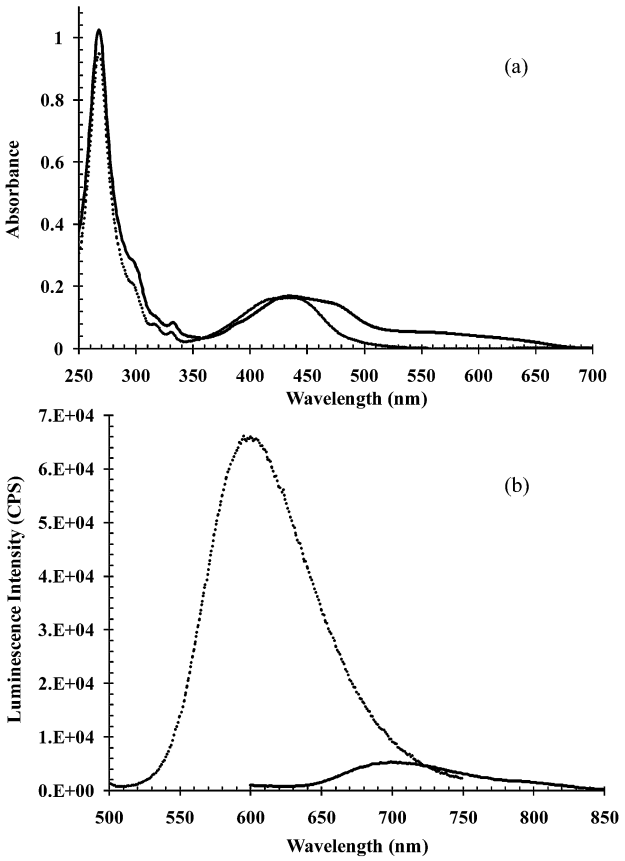


Fig. 2 Absorbance and luminescence spectra of $[Ru(tmphen)_3]^{2+}$ (dashed lines) and $[Os(tmphen)_3]^{2+}$ (solid lines). Complexes are 5.0 μM in water: (a) absorbance and (b) luminescence.

can be assigned to ligand-centered (^{1}LC) $\pi \rightarrow \pi^*$ transition. Moderately intense metal-to-ligand charge transfer (^{1}MLCT) $d \rightarrow \pi^*$ transitions are observed in the 400–500 nm region. Broad and weak absorptions at wavelengths higher than 600 nm corresponds to the ^{3}MLCT transition. Excitation of $[Os(tmphen)_3]^{2+}$ and $[Ru(tmphen)_3]^{2+}$ at any of its absorption wavelengths produced room-temperature photoluminescence maxima at 699 and 600 nm, respectively, in aqueous solution (Fig. 2). Similar to $[Os(bpy)_3]^{2+}$ and $[Os(phen)_3]^{2+}$, photoluminescence emission of $[Os(tmphen)_3]^{2+}$ was found to be solvatochromtic. We find a value for λ_{max} of 699 nm in water and 715 nm in acetonitrile. We did not determine photoluminescence quantum yields. Kober *et al.* report a value of 0.013 for $[Os(phen)_3]^{2+}$ in acetonitrile. It is clear from Fig. 2 that a considerable loss in luminescent intensity accompanies the change from $[Ru(tmphen)_3]^{2+}$ to $[Os(tmphen)_3]^{2+}$.

3.2 Electrochemical properties

Cyclic voltammetry studies of the ruthenium and osmium complexes were performed in a phosphate buffer solution. Table 1 shows the measured reduction potentials (corresponding to the M^{2+}/M^{3+} couple), peak-to-peak splitting (ΔE_p) and ratio of the magnitudes of the anodic and cathodic peaks (I_{pa}/I_{pc}). $E_{1/2}$ was taken to be a median of the potentials corresponding to the oxidation and reduction peak of the cyclic voltammograms shown in Fig. 3. In agreement with previous studies, complexes with ligands bearing electron-donating substituents ($-CH_3$) on 1,10-phenanthroline gave rise to low standard reduction potentials compared to 1,10-phenanthroline.[22] For the same ligand, the osmium complex exhibited much lower potential than that of ruthenium. The peak-to-peak splitting (ΔE_p) observed for all the complexes is 61 mV, close to the theoretical value of 59 mV expected for a reversible one-electron transfer.

Since $[Ru(tmpehn)_3]^{3+}$ and $[Os(tmphen)_3]^{3+}$ are the oxidants in the PFET application, their reduction potentials define an electrochemical limit for detectable analytes, and thus, the detection selectivity.

However, as shown in Table 2, the ability of M^{3+} to detect compounds is not simply related to the anodic peak potential of the analytes under investigation. For instance, $[Os(bpy)_3]^{3+}$ has shown to oxidize Fe^{2+}, ferrocene and DOP in 50 : 50 water–acetonitrile mixture. Based on the peak potentials in cyclic voltammetry of these analytes, oxidation would seem unlikely. Similarly, Jung *et al.* illustrated that despite high anodic peak potentials of nitrite, chlorite, bromide and phenol compared with $[Os(bpy)_3]^{3+/2+}$, phenol and bromide was unable to reduce $[Os(bpy)_3]^{3+}$ and yet nitrite and chlorite was able to (Table 2).[17] Based on the literature findings, Jung *et al.* concluded that the reactivity of analytes with $[Os(bpy)_3]^{3+}$ included a significant kinetic component.[17] Based on these observations, potential analytes were tested experimentally.

Under the experimental conditions described here, acetaminophen was oxidized by $[Ru(tmphen)_3]^{3+}$ and $[Os(tmphen)_3]^{3+}$, resulting in PFET signals, respectively. Based on the anodic peak potentials, $[Ru(tmphen)_3]^{3+}$ was expected to be reactive to dopamine and acetaminophen and $[Os(tmphen)_3]^{3+}$ only to dopamine. Despite greater peak potential of acetaminophen compared to $[Os(tmphen)_3]^{3+}$, signals were detected. We have not investigated this in detail, but we offer these observations: Nematollahi *et al.* work on electrochemical oxidation of acetaminophen in aqueous solutions, reported standard reduction potential of ~0.7 V (buffered at pH 7).[23] This was more positive than the standard reduction potential of $[Os(tmphen)_3]^{2+}$ (~0.413 V, pH 7), yet it gave

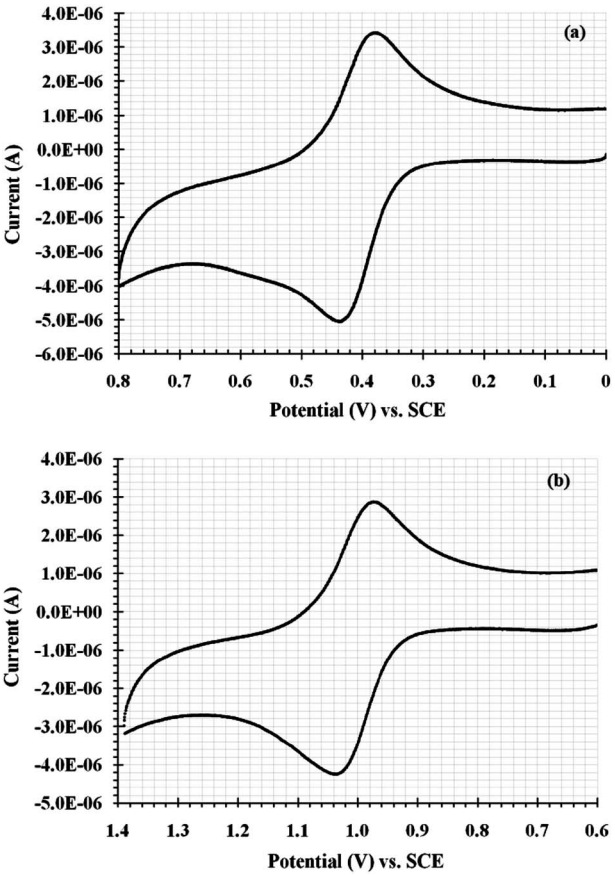


Fig. 3 Cyclic voltammograms (*vs.* sat. KCl Ag/AgCl reference electrode) of 0.4 mM solutions of $[Os(tmphen)_3]^{2+}$ (a) and $[Ru(tmphen)_3]^{2+}$ (b) in 0.10 M citric acid/phosphate buffer (pH 7.4). A 3 mm diameter glassy carbon electrode was used, the scan rate was 0.2 V s^{-1} and the electrolyte was 0.10 M NaCl.

Table 1 Spectroscopic and electrochemical data

Complex	λ_{abs}[a] (nm)		λ_{em}[a,b] (nm)	E°[c] (V)
	π–π^*	MLCT		
$Os(tmphen)_3(Cl)_2$	265	450, 550	699	0.413
$Ru(tmphen)_3(Cl)_2$	263	420, 450	600	1.07
$Ru(bpy)_3(Cl)_2$	285	427, 454	628	1.28

[a] 5 μM complex in 0.10 M phosphate buffer at pH 7, in a quartz cell of 1 cm path length. [b] Excitation at 488 nm. Excitation and emission slits were 10 and 15 nm, respectively. [c] 0.40 mM of complex. $M^{2+/3+}$ *vs.* SHE in 0.10 M citric acid/phosphate buffer (pH 7.4). A 3 mm diameter glassy carbon electrode was used, the scan rate was 0.2 V s^{-1} and the electrolyte was 0.10 M NaCl.

Table 2 Electrochemical properties of various analysis

Analytes	E_{pa} (V)	Reaction with $[Os(bpy)_3]^{3+}$	Reaction with $[Os(tmphen)_3]^{3+}$	Reaction with $[Ru(tmphen)_3]^{3+}$
Dopamine	0.617	Yes	Yes	Yes
Acetaminophen	0.7	Yes	Yes	Yes
Ferrocene carboxylic acid	0.771[a]	Yes		
Fe^{2+}	1.008[a]	Yes		
Br^-	1.33[a]	No		
Phenol	1.38[a]	No		
ClO_2^-	>1.5[a]	Yes		
NO_2^-	>1.5[a]	Yes		

[a] Anodic peak potential (E_{pa}) from Jung *et al.*[17] All potentials are reported *versus* SHE reference electrode.

PFET signal. In published work by Nematollahi *et al.*, the reaction mechanism for oxidation of acetaminophen in pH 7 solution was dependent on the acetaminophen concentration.[23]

As the concentration of acetaminophen was increased (1 mM to 10 mM), the peak current ratio (I_{pc}/I_{pa}) decreased and first cathodic peak potential shifted to negative direction.[23] Additionally, spectroscopic data from the isolated product was indicative of dimerization reaction between acetaminophen anion and NAPQI.[23] This was a second-order reaction; thus, its rate should diminish in homogenous solutions at low acetaminophen concentrations (nM to μM) used in the current study. This is consistent with the observed acetaminophen oxidation by $[Os[tmphen]_3]^{3+}$.

The standard reduction potential data presented in Table 1 show that the stability of the M^{3+} form of the complexes decreases in the order: $[Os(tmphen)_3]^{3+}$, $[Ru(tmphen)_3]^{3+}$, $[Ru(bpy)_3]^{3+}$. Significantly, this trend was shown in longer timescale experiments, when chemical oxidation was used to generate the M^{3+} form and the stability was monitored spectrophotometrically (following section). Chemically reversible voltammetric responses were observed for all complexes. However, it seems likely that the observed variations in M^{3+} stability for the different complexes in aqueous solution was due to increase in efficiency of the reversion of M^{3+} to M^{2+}.

3.3 Stability of the metal(III) oxidation state in aqueous solution

The relative stability of the M^{3+} complexes in aqueous solution after oxidation with lead dioxide was assessed by analyzing a mixture of each oxidized reagent by monitoring the absorbance at 436 nm (for Os(III) complex) and 750 nm (Ru(III) complex) as a function of time. A benefit of using lead dioxide is that lead dioxide is insoluble in water and subsequently can be removed by filtration.[30] As shown in Scheme. 1, the reaction between the M^{3+} and suitable reductant results in a luminescence signal. However, the M^{3+} species is also reduced by water, the rate of which is heavily dependent on the pH of the solution. This reaction has the detrimental effect of increasing the background signal of PFET as the pH is raised, which in turn is the determining factor in establishing detection limits of analyte under present investigation (acetaminophen).

Previously reported analytical applications based on PFET detection with either $[Ru(bpy)_3]^{2+}$ or $[Os(bpy)_3]^{2+}$ have involved on-line mixing of the M^{2+} complex and oxidant within a flow-analysis apparatus, where the period of time between solution mixing and detection is only a few second and controlled by the flow rate and the dimensions of the tubing and detector.[17] In the current study, off-line oxidation of M^{2+} complexes would extend the experiment time to minutes (4 min), which would result in poor reproducibility and sensitivity. Therefore, complexes used in current PFET system and analytes of interest, oxidized complexes were required to be stable and non-fluorescent at pH 7. Among the complexes studied, only $[Os(tmphen)_3]^{3+}$ met these requirements. Fig. 4 shows the stability of M^{3+} complexes in pH 7 buffered solution decreased in the order: $[Os(tmphen)_3]^{3+}$, $[Ru(tmphen)_3]^{3+}$, $[Ru(bpy)_3]^{3+}$. These results are consistent with the difference in the standard reduction potentials of the complexes also.

The pH dependent stability results (Fig. 5) indicated that, for the current PFET system, $[Os(tmphen)_3]^{3+}$ reagent would perform well below pH 3. Fig. 5 shows the reversion of Os^{3+} to Os^{2+} complex at various pHs. It is clear that the reversion increases as a function of pH. The increased reversion, and thus the background signal, can be due to hydroxide ion.[10] A pH < 7 is required to prevent more than 20% reversion of Os^{3+} complex over the time of 16 hours. A qualitative test was performed to visualize the effect of hydroxide ion on the stability of Os^{3+} complex. A concentrated solution of Os^{2+} complex (0.73 mM, pH 1) was used so that the reversion of Os^{3+} to Os^{2+} is visible. At this concentration, the dark-brown color of pre (Os^{2+}) and bluish-green color of post oxidized osmium complex (Os^{3+}) were highly visible and distinguishable. The Os^{3+} complex was

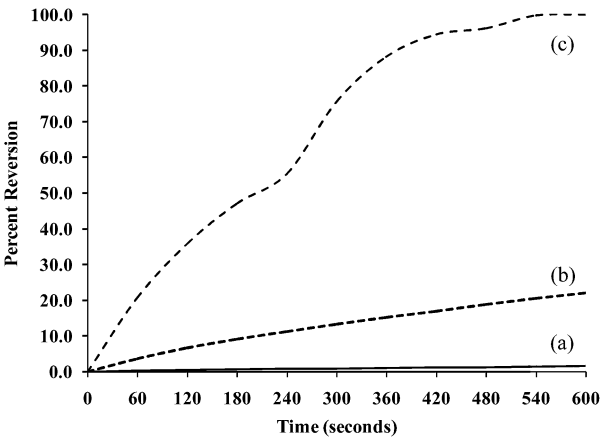


Fig. 4 Percent Reversion of $M(L)_3^{3+}$ to $M(L)_3^{2+}$. $M(L)_3^{3+}$ was prepared from a reaction of $M(L)_3^{2+}$ with PbO_2. Reversion was calculated by measuring absorbances of the Os(II) solution at 436 nm. Following oxidation of Os(II) complex, absorbance of the Os(III) solution was also measured at 436 nm and the difference in the absorbance was obtained to calculate percent reversion. Absorbance at 750 nm for Ru(II) and (III) complexes were measured to calculate its percent reversion. Concentration of complexes are 0.73 mM in aqueous solution at pH 7. (a) $[Os(tmphen)_3]^{3+}$, (b) $[Ru(tmphen)_3]^{3+}$ and (c) $[Ru(bpy)_3]^{3+}$.

observed to be stable for hours in pH 1 environment. When 100 μL of pH 10 buffer was added to Os^{3+} complex, the color reverted almost instantly to Os^{2+} with visible CL (data not shown). The stability of the reagents was also assessed in terms of their PFET intensity upon reaction with acetaminophen.

3.4 Linearity of acetaminophen

Fig. 6 shows the UV-Vis and photoluminescence spectra of $[Os(tmphen)_3]^{2+}$ and $[Ru(tmphen)_3]^{2+}$ complexes in buffered pH 7 solution before and after they are oxidized with PbO_2. Prior to oxidation, Os^{2+} and Ru^{2+} complexes were found to be fluorescent with maxima at 699 and 600 nm, respectively. Following oxidation, luminescence signal of the osmium solution was significantly reduced as compare to solution of Os^{2+} complex. To the contrary, luminescence signal of the ruthenium solution did not change. Following luminescence measurements of the oxidized solutions, absorption spectra of these solutions were collected. Absorption spectra of oxidized solutions of Os and Ru complexes were similar to that of Os^{3+} complex (diamond line in Fig. 6b) and Ru^{2+} (square line in Fig. 6a) complex, respectively. Thus, both spectroscopic measurements lead to the same conclusion: $[Os(tmphen)_3]^{2+}$ was completely oxidized, while $[Ru(tmphen)_3]^{2+}$ was not oxidized in pH 7 conditions. This allowed the extension of PFET system to $[Os(tmphen)_3]^{3+}$. As shown in Fig. 7, $[Os(tmphen)_3]^{3+}$ is non-luminescent and upon reacting with a reducing agent such as acetaminophen, the resulting Os(II) complex is luminescent.

To investigate the performance of $[Os(tmphen)_3]^{3+}$ as luminescent probe for the quantitative detection of acetaminophen, the emission spectra of $[Os(tmphen)_3]^{2+}$ in the presence of different concentrations of acetaminophen in 0.05 M phosphate buffer at pH 7.0 were measured at room temperature (Fig. 7). As expected, upon the addition of reducing agent to the solution of Os^{3+} complex, the luminescencent Os^{2+} complex was generated. Interestingly, the luminescence intensity of Os^{2+} complex was quantitatively proportional to the concentration of acetaminophen from 3.02 μg L^{-1} to 2267.4 μg L^{-1} (Fig. 8). The detection limit for acetaminophen was 1.5 μg L^{-1}. In comparison with the results from reported methods, approximately a 1–5 order of magnitude lower detection limit was achieved.[24–29] The comparison is shown in Table 3. These results indicate that our new $[Os(tmphen)_3]^{2+}$ complex-based luminescence probe is more sensitive with lower detection limit for the quantitative detection of acetaminophen in pH 7 aqueous solutions.

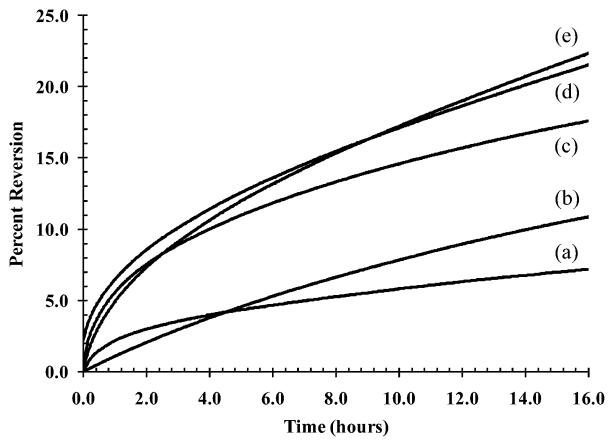


Fig. 5 Percent reversion of $[Os(tmphen)_3]^{3+}$ to $[Os(tmphen)_3]^{2+}$. Os(III) was prepared from a reaction of Os(II) with PbO_2. Reversion was calculated by measuring absorbances of the solution at 436 nm. Concentration of osmium complexes 0.73 mM in aqueous solution at various pHs. (a) pH 3, (b) 4, (c) 5, (d) 6 and (e) 7.

3.5 Reliability of the method

3.5.1 2x1D-SPE: determination of pH and percentage of methanol in the wash and elution steps. Retention of the analyte in reversed-phase SPE is controlled by two key factors; pH and concentration of the eluting solvent. Increase in the concentration of the eluting solvent, decreases the retention of the analyte. Dependence of pH on analyte retention is correlated with the nature of the compounds. This is the case for acetaminophen (pK_a 9.5). Retention of acetaminophen on the reversed-phase HLB column is significantly different in an acidic or basic environment. At pH value lower than the pK_a, acetaminophen is present in unionized form, and thus will exhibit higher retention. Similarly, at pH value higher than the pK_a, acetaminophen is ionized, and have low retention. A wash step at pH value lower than pK_a would remove acidic and neutral interferences while ensuring that the acetaminophen remains adsorbed. Elution with pH greater than pK_a should then extract acetaminophen along with additional analytes in urine with similar pK_a values. Eluate is loaded onto another reversed-phase HLB column from which acetaminophen is extracted as the function of methanol concentration. This should enable us to isolate acetaminophen from a complex urine matrix with minimum interferences.

Determination of pH and percentage methanol was simplified by preparing acetaminophen samples in a saline solution instead of a urine matrix. Acetaminophen was not eluted in the wash steps containing pH 7, 8, and 9.2 buffered solutions, respectively. Acetaminophen was eluted with 98.88% recovery (14 948 μg L^{-1}) in pH 10.8 solution.

Table 4 shows the results of acetaminophen elution study with increasing methanol concentration (0 to 30%). In the first elution (elute 1, 0.25% methanol), acetaminophen began to elute with 11.16% (1687 μg L^{-1} acetaminophen) recovery. As the concentration of methanol increased, so did the percent recovery. In order to obtain 100% recovery of acetaminophen, >30% of methanol was needed. Based on these results, we chose pH 10.8 as the elution solvent for the first 1D-SPE. The pH of this eluate was readjusted to 7.0 with concentrated hydrochloric acid and then loaded on the second column, from which, acetaminophen was eluted with 0.25% methanol. This percentage of methanol was chosen because not only it eluted 28× the concentration of AP normally found in urine (10.69 μg L^{-1}–59.72 μg L^{-1}) but with minimum co-elution of interferences from urine. UV-Vis spectra of pH dependence study is shown in Fig. S2.†

The complete 2x1D-SPE steps are as follows: load 2 mL of which 400 μL is urine and 1600 μL is pH 7 buffer (refer to the

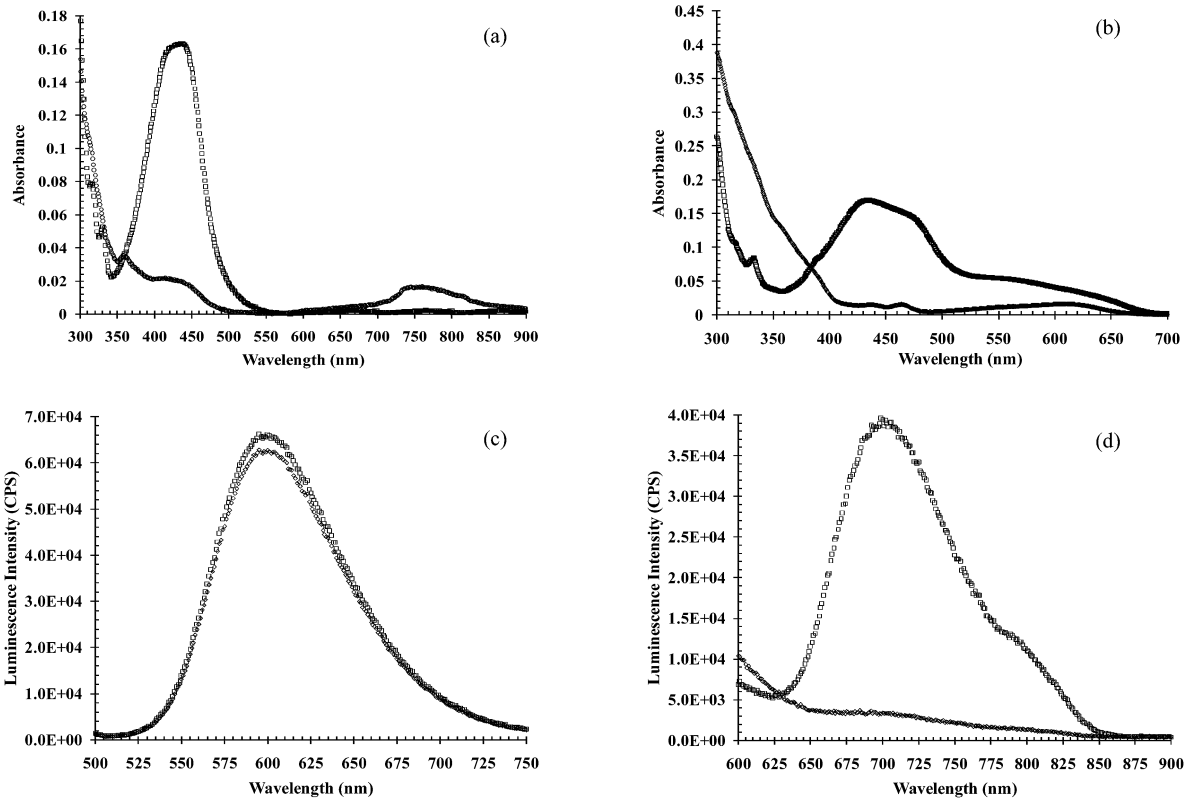


Fig. 6 Absorbance and luminescence spectra of $[Ru(tmphen)_3]^{2+}$ and $[Os(tmphen)_3]^{2+}$ before and after reaction with solid PbO_2. Squares (□) show spectra of the M^{2+} complexes and diamonds (◇) show spectra of the M^{3+} complexes. Concentration of complexes is 8.78 μM in water: (a) $[Ru(tmphen)_3]^{2+/3+}$ absorbance and (b) $[Os(tmphen)_3]^{2+/3+}$ absorbance (c) $[Ru(tmphen)_3]^{2+/3+}$ luminescence, and (d) $[Os(tmphen)_3]^{2+/3+}$ luminescence.

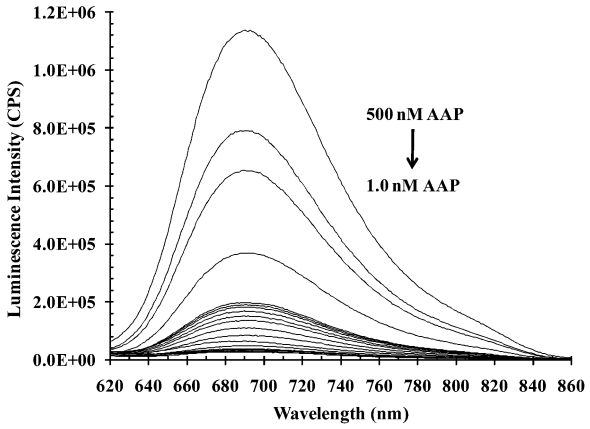


Fig. 7 Luminescence spectra of $Os(tmphen)_3]^{2+}$ in the presence of different concentrations of acetaminophen in 0.10 M phosphate buffer at pH 7.0. Concentration of $[Os(tmphen)_3]^{2+}$ is 8.78 μM.

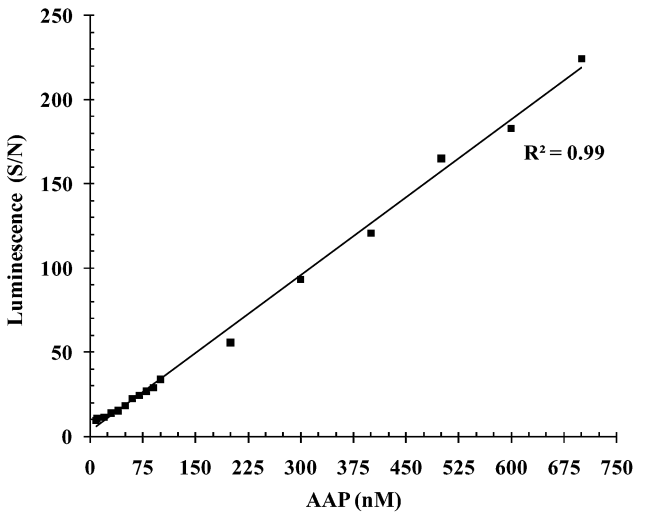


Fig. 8 Luminescence signal-to-noise ratios of $Os[(tmphen)_3]^{2+}$ generated from reaction between $Os[(tmphen)_3]^{3+}$ and analyte; acetaminophen (■) in 0.10 M phosphate buffer at pH 7.0. Concentration of $[Os(tmphen)_3]^{2+}$ is 8.78 μM.

sample collection and preparation section); first wash with 2 mL pH 7 buffer; second wash with 2 mL of pH 8.0 buffer; thirst wash with 2 mL pH 9.2 buffer; elute with 2 mL pH 10.8 buffer. Readjust the pH of the eluate to 7.0 with hydrochloric acid and load it on the second column. Elute with 2 mL of 0.25% methanol. Evaporate the eluate to dryness at 60 °C under a gentle stream of nitrogen and reconstitute with 2 mL of 0.1 M pH 7.0 buffer solution. With this protocol, we were able to obtain clean extracts as well as consistent recoveries of acetaminophen from urine matrix.

Table 3 Method comparison for the determination of acetaminophen

Method	Detection Limit (μg L^{-1})	Reference
This method	1.5	
LC-MS/MS	0.3	31
HPLC	6.0	24
Flow injection-spectrophotometric	10.6	26
Fluorescence	10.6	27

Table 4 Wash-elute study to determine dependence of percentage of methanol on the elution of acetaminophen. Seven columns were loaded with an aliquot of 2 mL sample solution (15.18 μg mL^{-1} of acetaminophen in pH 7.0 phosphate buffer solution). The acetaminophen was eluted from each column with 2 mL of methanol–water mixtures of increasing methanol concentration (0, 0.25, 0.5, 1, 5, 10 and 30% methanol), respectively ($n = 3$)

Column	% Methanol	% Recovery
1	0.25	11.2
2	0.5	15.9
3	1.00	18.7
4	5.00	26.10
5	10.0	34.5
6	30.00	101.00

The specificity criteria was met by the 2x1D-SPE procedure which reduced the $[Os(tmphen)_3]^{2+}$ luminescence signal below the limit of detection due to interferences from urine.

3.5.2 Precision and accuracy: urine analysis. To determine the accuracy of the method we analyzed urine samples with three levels of acetaminophen concentrations: low (40.4 μg L^{-1}), medium(120.0 μg L^{-1}) and high (360.0 μg L^{-1}). These concentrations were chosen to reflect the broad spectrum of urinary matrix. These samples were analyzed in native (non-spiked) and spiked conditions. The non-spiked urine samples contained no native acetaminophen, thus the measured signal was due to the reduction of Os^{3+} (non-luminescent) to Os^{2+} (luminescent) by the interferences from urine that co-eluted with acetaminophen. Thus the luminescence signal measured from the spiked samples were subtracted from the native samples before calculation. The results are summarized in Table 5 and Fig. S3.† For the low-spiked concentration, the mean calculated accuracy (percent recovery) was 97.1% (94.3%–106.2%). The mean accuracy (percent recovery) calculated from medium and high concentration samples were, 101.5% (88.1%–107.3%) and 90.9% (88.2%–98.4%) respectively. The precision data obtained from these spiking experiments have a RSD of 7.0% for the low concentration and RSD's of 11.6% and 6.5% for medium and high concentration, respectively. The accuracy and precision data obtained from our method was comparable to the reported HPLC-MS/MS method for the analysis of urine samples with two different spiking level of acetaminophen; low (109.7 μg L^{-1}) and high (548.5 μg L^{-1}).[34] The mean accuracy (precision) from high and low concentration samples was 98.4% (4.5%) and 100.2% (2.3%), respectively. Furthermore, as can be seen in the results of urine matrix, the highest acetaminophen level determined in these samples was more than a factor of 3 above the LOQ.

Table 6 Precision and accuracy calculated from analysis of pharmaceutical sample ($n = 3$)

	Spiking level		
	Low	Medium	High
Spiked concentration (μg L^{-1})	75.60	151.2	226.8
Concentration from tablet (μg L^{-1})	75.60	75.6	75.6
Expected total concentration (μg L^{-1})	151.2	226.8	302.4
Total concentration measured (μg L^{-1})	147.5	231.0	287.6
Spiked conc. calculated (μg L^{-1})			
Mean	73.75	154.1	215.7
RSD (%)	1.2	2	3.1
Accuracy (%)	97.55	101.9	95.11
Content (mg per tablet)	487.8	509.3	475.5
Label (mg per tablet)	500	500	500
Δ (%)	−2.4	1.9	−4.9

3.5.3 Pharmaceutical formulations analysis. The proposed method has been applied to the determination of acetaminophen in a commercial pharmaceutical formulation ($n = 3$). The results are summarized in Table 6 and Fig. S4.† It can be seen from the results obtained utilizing the proposed PFET method, as well as from the respective standard deviations, that the relative errors between the experimental results and the nominal value specified by the pharmaceutical company are small. In addition, the recovery on the basis of this work was between 95.11% and 101.85%. The results suggested that the proposed method was reliable and sensitive enough for the quantification of acetaminophen in real pharmaceutical samples.

Table 5 Precision and accuracy calculated from analysis of urine sample ($n = 3$) with varying acetaminophen concentrations

	Spiking level		
	Low	Medium	High
Spiked conc. (μg L^{-1})	40.41	120.0	360.0
Spiked conc. measured (μg L^{-1})			
Mean	39.24	121.8	327.2
Range	38.12–42.92	105.7–130.8	317.5–354.2
RSD (%)	7.0	11.6	6.5
Accuracy (%)	97.1 (94.0–106.2)	101.5 (88.1–109.2)	90.9 (86.3–98.4)

4. Conclusions

A new $[Os(tmphen)_3]^{3+}$ reagent for luminescence detection was prepared by dissolving the reagent in pH 7 aqueous solution (containing 0.05 M phosphate buffer) followed by oxidation with PbO_2. The new reagent was more stable than conventional aqueous and non-aqueous luminescence reagents, and allowed extended periods of analysis without the need for preparation of fresh reagent, thus overcoming a limitation of $[Ru(bpy)_3]^{3+}$ as PFET reagent. Of all reagents, $[Os(tmphen)_3]^{3+}$ was found to be most suitable as PFET reagent in pH 7 conditions. This is a clear indication that the absence of a large background luminescence resulting from $[Os(tmphen)_3]^{3+}$ decomposition is the determining factor in establishing detection limits. The PFET reagent, as demonstrated in this work, is highly suited to the quantitative determination of acetaminophen in standard solutions. The detection limit (1.5 μg L^{-1}) of acetaminophen was lower than the number of previously reported methods.[24–29]

Additionally, we have developed novel, robust and selective 2x1D-Solid Phase Extraction (2x1D-SPE) method to determine acetaminophen in urine samples in a wide concentration range. With the SPE method, high and consistent recoveries were obtained. pH and methanol concentration were optimized simultaneously to isolate acetaminophen from urine matrix with minimum interferences. The proposed method has also proved to be robust and sensitive for quantification of acetaminophen in pharmaceutical preparations. As also previous concluded by Jung *et al.*[17] we found the major limitations of the present technique is nonspecific reduction of the reagent to the fluorescent form. Major advantages are day-to-day reproducibility, sensitivity, and the use of this very stable osmium reagent will enhance the exploration PFET application in pure aqueous (pH 7) media.

Abbreviations

PFET	Photoluminescence-following electron-transfer
CL	Chemiluminescence
ECL	Electrochemiluminescence
MLCT	Metal-to-ligand charge transfer
LC	Ligand-centered
tmphen	3,4,7,8-Tetramethyl-1,10-phenanthroline
bpy	Tris (2,2′-bupyridine
SPE	Solid phase extraction

Acknowledgements

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