

# Using 5,10,15,20-tetra(4-nitrophenyl)porphyrin as a fluorescent chemosensor to determine Ru<sup>3+</sup>

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Received 11 Jan 2012

Accepted 12 Jun 2012

**ABSTRACT:** The 5,10,15,20-tetra(4-nitrophenyl)porphyrin (TNPP) has been synthesized using the reaction of pyrrole and 4-nitrobenzaldehyde in the presence of propionic acid. The TNPP was used as a new fluorescent chemosensor to determine Ru<sup>3+</sup> with higher selectivity than other ions such as Hg<sup>2+</sup>, CH<sub>3</sub>Hg<sup>+</sup>, Ag<sup>+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, Ni<sup>2+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, or Zn<sup>2+</sup>. The response of the new chemosensor is reported in the form of fluorescence quenching, which is attributed to the metalloporphyrin formation of Ru-TNPP complex with 1:1 stoichiometric ratio ( $K_{\text{stab}} = 2.04 \times 10^8$ ). The synthesized fluorescence chemosensor can be used to determine Ru<sup>3+</sup> with a detection limit of 0.89 μM (90 ppb) and a linear quantification range between 3 and 25 μM. Therefore, the synthesized chemosensor could be used as a new fluorescence chemosensor for the quantitative analysis of Ru<sup>3+</sup> with satisfactory results.

**KEYWORDS:** porphyrin derivative, porphyrin complex of Ru<sup>3+</sup>, ion selectivity

## INTRODUCTION

Among several metal cations, ruthenium ion is widely used in many applications including catalyst<sup>1,2</sup>, dye-sensitized solar cell<sup>3–5</sup>, and in medicine as an anti-cancer agent<sup>6–8</sup>. However, extensive use of ruthenium ion could lead to accumulation of wastes harmful to the environment, as ruthenium ion exhibits various forms of toxicity<sup>9</sup>. All ruthenium-containing compounds are considered toxic because they can stain skin strongly, and ingested ruthenium can also be retained strongly in bone<sup>10</sup>. Hence, the determination of ruthenium ion in an environment has gained importance due to environmental toxicity concerns. Various methods to determine Ru<sup>3+</sup>, such as chromatography, atomic absorption, and ICP-MS have been previously reported in the literature<sup>11–14</sup>. However, these methods require either sophisticated operation or difficult sample preparation. Therefore, the development of simpler methods to determine ruthenium ion is crucial and remains an attractive research topic in environmental toxicology and analytical chemistry.

Nowadays, the design and development of highly selective and fluorescent sensors for transition-metal cations with high selectivity have been widely explored due to the high sensitivity and selectivity of

fluorescence method<sup>15,16</sup>. The porphyrin macrocycles have been used as fluorophore owing to their high absorption coefficient in the visible range, tunable fluorescence emission, large Stoke shift, which can minimize fluorescence background and high photo- and chemical-stability<sup>17–19</sup>. In addition, porphyrin metallation can be used as a method to determine metal cations when emission properties of metallo- and free base-porphyrin are significantly different<sup>20</sup>.

Herein, 5,10,15,20-tetra(4-nitrophenyl)porphyrin (TNPP) was synthesized and then used as a fluorescent chemosensor for the determination of Ru<sup>3+</sup>. To the best of our knowledge, TNPP has been synthesized by various methods<sup>21–23</sup> but it has never been used as a tool to determine the amount of any ions before. Consequently, it would be beneficial to use TNPP as a new fluorescent chemosensor to determine Ru<sup>3+</sup>.

## MATERIALS AND METHODS

### Reagents and chemicals

All reagents and solvents were commercially available and used as received without further purification. The standard solutions of metal cations were prepared from a standard solution 1000 ppm atomic absorption spectroscopy (AAS) grade except mercury ion

(Hg(II)) and methylmercury ion ( $\text{CH}_3\text{Hg(I)}$ ), which were used from solid chloride salt.

### Apparatus

The  $^1\text{H}$ -NMR spectra were recorded on an INNOVA VARIAN NMR spectrometer 400 MHz spectrometer with chemical shifts (in ppm) relative to  $\text{CDCl}_3$ . The FT-IR spectra were obtained by Perkin Elmer system 2000 Fourier transform infrared spectrometer using KBr pellets in the range of  $4000\text{--}400\text{ cm}^{-1}$ . Electrospray ionization mass spectrometry (ESI-MS) was performed on a Bruker microTOF mass spectrometer using tetrahydrofuran (THF) as a carrier solvent. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer.

### Synthesis of 5,10,15,20-tetra(4-nitrophenyl)-porphyrin (TNPP)

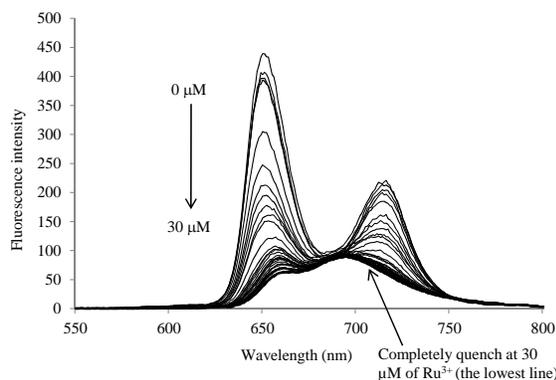
The TNPP was synthesized according to a previously reported protocol<sup>24</sup>. In brief, 4-nitrobenzaldehyde and pyrrole were heated to reflux in the presence of propionic acid. TNPP was obtained in 20% yield and was characterized by spectroscopic techniques.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.82 (s, 8H, pyrrole-H), 8.66 (d, 8H,  $J = 8.8\text{ Hz}$ , ArH), 8.40 (d, 8H,  $J = 8.8\text{ Hz}$ , ArH). ESI-MS: ( $m/z$ ) 795.7 [ $\text{M}+\text{H}$ ] $^+$ . FT-IR (KBr,  $\text{cm}^{-1}$ ): 846 (s), 864 (s), 963 (s), 1223 (w), 1344 (s), 1400 (w), 1516 (s), 1595 (s), 1679 (w), 3313 (w).

### General spectroscopic method

Methanol and THF were used as solvents for spectroscopic experiments without any further purification. The stock solution of  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^+$  were prepared from their corresponding chloride salts. The stock solutions of  $\text{Ru}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  were prepared from 1000 ppm AAS standard solution.

The stock solution of TNPP was prepared by dissolving TNPP in THF. For 1 mM stock solutions of metal cations, an appropriate amount of each metal cation was dissolved in methanol and the volume was adjusted to 100 ml in a volumetric flask. Then, all solutions were further diluted to 0.1 mM.

Fluorescence spectra were recorded at room temperature. The complex solution of Ru-TNPP was prepared by adding 250  $\mu\text{l}$  of 1 mM stock solution of  $\text{Ru}^{3+}$  to 2.5 ml solution of 0.1 mM TNPP. Then, volume was adjusted to 10 ml by THF. For the fluorescence titration, 10  $\mu\text{l}$  aliquots of  $\text{Ru}^{3+}$  solution (from 0.1 mM and 1 mM of  $\text{Ru}^{3+}$  solution in methanol) were added to 2.5 ml TNPP solution (0.1 mM in THF). The excitation and emission wavelength for



**Fig. 1** Fluorescence titration spectra of TNPP (25  $\mu\text{M}$ ) upon the addition of  $\text{Ru}^{3+}$  at different concentrations (0–30  $\mu\text{M}$ ).

all measurements were fixed at 430 and 651 nm, respectively. The UV-Vis spectroscopic measurements were performed using a Perkin Elmer Lambda 35 UV-Vis spectrophotometer at room temperature. All spectroscopic measurements were conducted using quartz cuvettes with 1 cm path length.

## RESULTS AND DISCUSSION

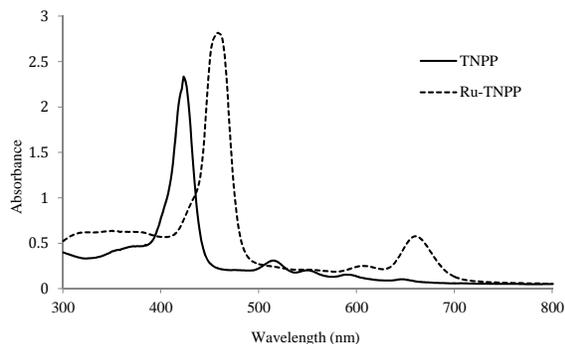
### Fluorescence spectra and fluorescence titration

The fluorescence spectrum of TNPP shows a sharp band with a maximum at 651 nm. When  $\text{Ru}^{3+}$  was added to the TNPP solution, fluorescence quenching was observed. In order to determine the amount of  $\text{Ru}^{3+}$  required to completely quench the fluorescence signal from TNPP, the fluorescence titrations were performed (Fig. 1). It was found that the fluorescence of TNPP was almost completely quenched when one equivalent of  $\text{Ru}^{3+}$  was added, indicating the formation of TNPP complex.

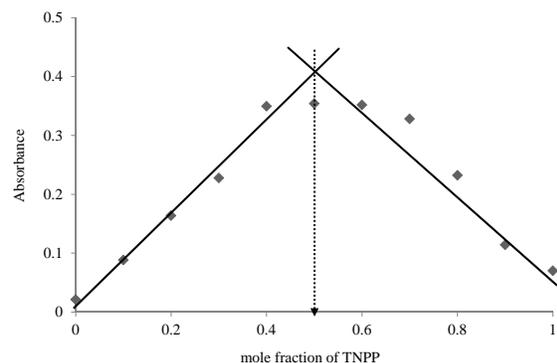
To determine the linear range and detection limit, the fluorescence quenching efficiency ( $I_0/I$ ) values of TNPP were plotted against concentrations of  $\text{Ru}^{3+}$ . The plot exhibited a linear relationship between  $I_0/I$  and concentration of  $\text{Ru}^{3+}$  over the range of 3–25  $\mu\text{M}$ . The regression equation is expressed as follows:  $y = 0.2362x + 0.6752$  ( $R^2 = 0.9849$ ), where  $y$  = fluorescence quenching efficiency and  $x$  = concentration of  $\text{Ru}^{3+}$ . The limit of detection (defined as  $y_b \pm 3s_b$ ) for quantifying  $\text{Ru}^{3+}$  by TNPP was determined to be 0.89  $\mu\text{M}$ , which is equal to 89.95 ppb<sup>25,26</sup>.

### Absorption spectra

Absorption spectra of TNPP in the absence and in the presence of  $\text{Ru}^{3+}$  were recorded (Fig. 2). The absorption spectrum of TNPP exhibits a Soret band



**Fig. 2** UV-Vis spectra of TNPP (25  $\mu\text{M}$ ) in the absence and in the presence of  $\text{Ru}^{3+}$  (25  $\mu\text{M}$ ).

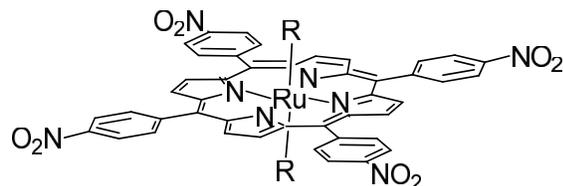


**Fig. 3** Job plot for the determination of stoichiometry of Ru-TNPP complex.

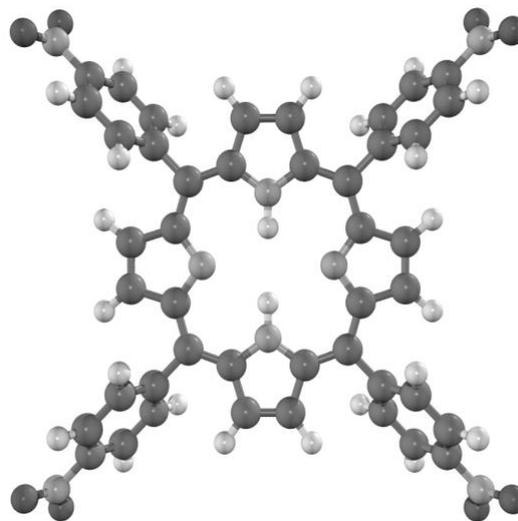
at 423 nm and Q band at 513 nm. Upon addition of  $\text{Ru}^{3+}$ , the absorption spectrum displays a shift of Soret band and Q band to 453 nm and 601 nm, respectively. These spectroscopic characters indicate the formation of Ru-TNPP complex.

To determine the stoichiometry of Ru-TNPP complex, Job's method was conducted<sup>27</sup>. The plot between absorbance and mole fractions of TNPP at 455 nm is shown in Fig. 3. It was found that the stoichiometric ratio of Ru-TNPP complex was 1:1. Moreover, from Job's method, the stability constant was calculated to be  $2.04 \times 10^8$  for the Ru-TNPP complex<sup>27</sup>. Based on these results, a possible structure of Ru-TNPP complex was proposed, where tetrapyrrolic cavity of the TNPP provides a coordination site for  $\text{Ru}^{3+}$  and, due to the literature reviews<sup>28,29</sup>, the solvent molecules such as MeOH and/or THF can provide 6 coordinations with  $\text{Ru}^{3+}$  on the axial position (Fig. 4).

To understand the occurrence of Ru-TNPP complex, the quantum chemical calculation of TNPP was carried out. The optimized structure of TNPP us-



**Fig. 4** A possible structure of Ru-TNPP complex (R = MeOH and/or THF).

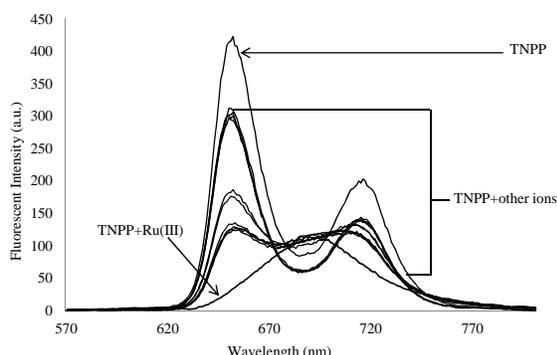


**Fig. 5** The optimized structure of TNPP.

ing GAUSSIAN03 program is shown in Fig. 5. The calculation uses density functional theory at B3LYP level of theory and the 6-31G\* basis set. From the optimized structure, the diameter of TNPP cavity is 4.2 Å. Comparing to the diameter of  $\text{Ru}^{3+}$ , which is (1.64 Å)<sup>30</sup>. One can see that the size of  $\text{Ru}^{3+}$  is small enough to fit in the tetrapyrrolic cavity of the TNPP. These results support the occurrence of Ru-TNPP complex via addition of  $\text{Ru}^{3+}$  to the solution of TNPP.

### Selectivity

To investigate the selectivity of TNPP ligand for  $\text{Ru}^{3+}$  over other metal cations, some cations ( $\text{Hg}^{2+}$ ,  $\text{CH}_3\text{Hg}^+$ ,  $\text{Ag}^+$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ ) were analysed and the fluorescence responses of these cations were compared with that of  $\text{Ru}^{3+}$ . It was found that fluorescence quenching response of TNPP in the presence of  $\text{Ru}^{3+}$  is greater than any other cations (Fig. 6). Moreover, interferences from other cations were also studied. The addition of aforementioned metal cations into the solution of TNPP



**Fig. 6** The fluorescence spectra of TNPP (25 μM) and TNPP in the presence of various metal cations, which are Hg(II), CH<sub>3</sub>Hg(I), Ag(I), Cd(II), Cr(III), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) at the concentration of 25 μM at the stoichiometric ratio of 1:1.

**Table 1** Fluorescent sensing of Ru<sup>3+</sup> by TNPP (25 μM) in the presence of interfering cations.

Ion	Molar ratio of interference ion : Ru(III)						
	0.007:1	0.01:1	0.02:1	0.1:1	0.5:1	1:01	10:01
Hg(II)	–	–	–	–	+	+	+
CH <sub>3</sub> Hg(I)	–	–	–	–	+	+	+
Ag(I)	–	–	–	–	+	+	+
Co(II)	–	–	–	+	+	+	+
Cd(II)	–	–	–	–	+	+	+
Ni(II)	–	–	–	+	+	+	+
Cr(III)	–	–	–	–	+	+	+
Fe(III)	–	–	–	–	+	+	+
Cu(II)	–	–	–	+	+	+	+

+ = Interfere  
 – = Not interfere

containing Ru<sup>3+</sup> resulted in negligible change of fluorescence intensity at low concentration, whereas all cations could interfere with the analysis of Ru<sup>3+</sup> at high concentration (Table 1). However, at the ratio of 0.1:1 (interference ion:Ru<sup>3+</sup>), the determination of Ru<sup>3+</sup> by TNPP was strongly interfered. From HSAB theory<sup>30</sup>, the results might be due to the borderline acid character of these cations and the size of these cations that allow them to be coordinated by the TNPP. Besides, it is well known that porphyrins can form stable complex with metal ions such as Zn, Cu, Co, or Ni which are the main interference ions. Although there are some interferences for using TNPP as a fluorescent chemosensor, the interferences from selected metal cations are negligible at low concentration of interference ions. Based on the results presented here, using TNPP as fluorescence chemosensor could be a new approach for determining Ru<sup>3+</sup> with low limit of detection.

**Table 2** The determination of Ru<sup>3+</sup> in synthetic waste water samples.

Sample	Ru <sup>3+</sup> spiked (μM)	Ru <sup>3+</sup> concentration (μM) (mean ± SD, n = 3)	Recovery (%)
1	5	4.62 ± 0.37	92.4
2	10	9.44 ± 0.21	94.4
3	15	15.74 ± 0.18	104.9

The synthesized porphyrin chemosensor was used in the determination of Ru<sup>3+</sup> in the synthetic samples in order to display how TNPP work. Different samples were prepared by adding other ions such as Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Ni<sup>2+</sup> in order to mimic the actual waste water content from industries<sup>31,32</sup>. Aliquots of different concentrations of Ru<sup>3+</sup> were added to the prepared water sample (having other ions 100 times lower than Ru<sup>3+</sup> concentration). These samples were then analysed by treating with the synthesized TNPP followed by the fluorescence measurement. The recoveries of spiked Ru<sup>3+</sup> (compared to the blank) determined by TNPP displayed satisfactory results (Table 2).

**Conclusions**

In summary, TNPP was synthesized and used as a new fluorescence chemosensor for the analysis of Ru<sup>3+</sup>. The complexation between TNPP and Ru<sup>3+</sup> quenches the fluorescence of TNPP at 651 nm. The stoichiometry of the complex between TNPP and Ru<sup>3+</sup> was determined to be 1:1 by Job’s method with a stability constant of 2.04 × 10<sup>8</sup>. Although the selectivity of TNPP to Ru<sup>3+</sup> at high concentration level of interference ions is poor, the fluorescent chemosensor TNPP is still a convenient way to determine Ru<sup>3+</sup> in water samples without sophisticate method in case of low level of interference ions. Moreover, the results support the use of TNPP as a new approach for the fluorescence quantification of Ru<sup>3+</sup>.

*Acknowledgements:* The authors would like to thank the Department of Chemistry, Kasetsart University and the Centre of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education for financial support. The authors would also like to thank the Faculty of Science, Kasetsart University for Bilateral Research Collaboration grant for this study. Besides, I wish to express my sincere gratitude to Dr Pitak Chuawong, Dr Sornnarin Bangkedphol, and Miss Kanokwan Kongpatpanich for their helpful assistance.

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