การวิเคราะห์เชิงแสงฟลูออเรสเซนท์ของไอออนไซยาไนด์โดยใช้ระบบของ เมโซ- เททระฟีนิลพอร์ไฟริน/ เมโซ-เททระฟีนิลพอร์ไฟริน โคบอลท์ (II)

FLUORIMETRIC DETERMINATION OF CYANIDE ION (CN^-) BY *MESO*-TETRAPHENYLPORPHYRIN/ *MESO*-TETRAPHENYLPORPHYRIN COBALT (II) SYSTEM

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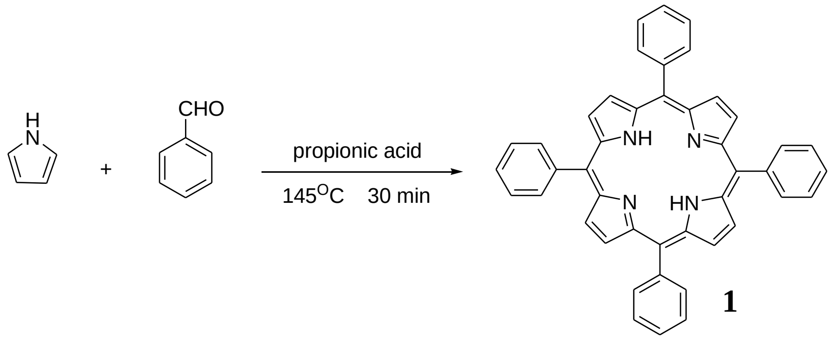
บทคัดย่อ: เมโซ–เททระฟีนิลพอร์ไฟริน (**1**) สังเคราะห์ได้โดยปฏิกิริยา Rothemunt and Alder-longo โดยการรีฟลักซ์ระหว่างเบนซัลดีไฮด์และพีร์โร สารเชิงซ้อนเมโซ–เททระฟีนิลพอร์ไฟริน โคบอลท์ (II) (**2**) สังเคราะห์ได้โดยการรีฟลักซ์ **1** และโคบอลท์คลอไรด์ ($CoCl_2$) ในสารละลาย DMF โดยที่สาร **1** และ **2** สามารถใช้เป็นตัวรับรู้เชิงแสงฟลูออเรสเซนท์ของไอออนไซยาไนด์ (CN^-) โดยอาศัยหลักการแข่งขันการแทนที่ของสาร **1** และ **2** ในสารละลายผสมของน้ำและเททระไฮโดรฟิวแรนสาร **1** จะให้แสงฟลูออเรสเซนท์ความเข้มสูงที่ความยาวคลื่น 650 นาโนเมตร ซึ่ง ความเข้มนั้นสามารถลดลงได้โดย **2** และสามารถกลับคืนมาได้อีกครั้งโดย CN^- สำหรับการตอบสนองต่อ CN^- นั้นสามารถสังเกตได้ง่ายเนื่องจากเกิดการเปลี่ยนแปลงสีของสารละลายอย่างชัดเจนแม้ที่ความเข้มข้น 1 μM ความสามารถในการวิเคราะห์ด้วยระบบนี้แสดงสมการเส้นตรง $y = 233.7 + 5.526x$ ($R^2 = 0.981$) ในช่วงความเข้มข้น 3 ถึง 15 mM โดยที่ y เป็นความเข้มของแสงฟลูออเรสเซนท์และ x คือความเข้มข้นของ CN^- โดยมีขีดจำกัดการวิเคราะห์เชิงปริมาณเป็น 2.25 mM และในการทดลองนี้ได้มีการศึกษาถึงไอออนที่เป็นตัวรบกวนในการตรวจวัดด้วย

Abstract: Meso-tetraphenylporphyrin (**1**) was synthesized by Rothemund and Alder-Longo reaction from refluxing between benzaldehyde and pyrrole in propionic acid. The meso-tetraphenylporphyrin cobalt (II) (**2**) was also prepared by refluxing **1**with $CoCl_2$ in DMF solution. **1** and **2** were used for fluorescent sensing of cyanide ion (CN^-) based on the competitive-displacement strategy between **1** and **2**. In THF-water solution, **1** emits strong fluorescence at 650 nm but it was quenched by **2** then restored by CN^-. The recognition of CN^- could be easily achieved due to the significant color change by CN^- even at low concentration of 1 μm. The analytical performance of this system exhibited the linear equation $y = 233.7 + 5.526x$ ($R^2 = 0.981$) in the concentration range of 3.0-15.0 mM where y is fluorescent intensity and x is the concentration of CN^- in the practically usable range and the limit of quantitation was 2.25 mM. The interferences of this system were also studied.

Introduction: Cyanide ion (CN^-) is one of the important inorganic anions which have made a great effect on both environment and human body. CN^- has been widely used in electroplating factories of gold, silver, copper and cadmium, dressing plant and the steel heat treatment factory. The concentration of CN^- environmental water sample is regulated strictly by its toxicity. The standard of CN^- concentration in the water pollution control is 1 mg/l. Therefore, it is necessary to use a method that determines concentration of very small amounts of CN^-. Several methods, for example, chromatographic method, amperometric method and ion selective electrode (ISE) can be used for determining of CN^- but they require sophisticated operation and time-consuming [1].

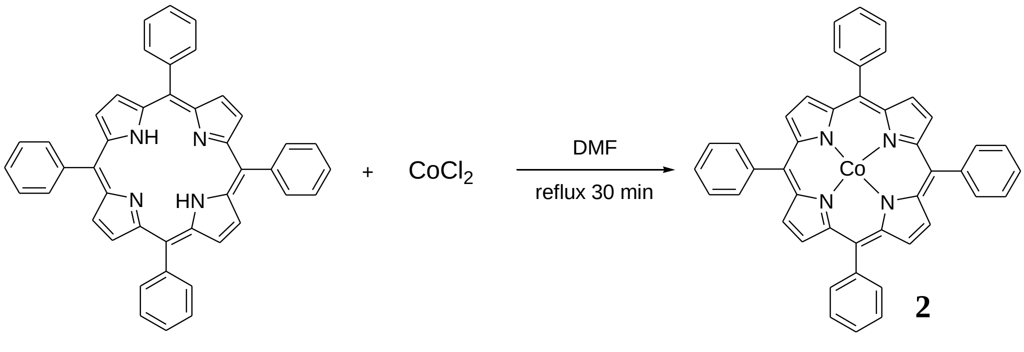
Porphyrins and metalloporphyrins are attractive candidate owing to their fluorescent properties. It was found that in organic medium, *meso*-tetraphenylporphyrin **(1)** could be quenched by the presence of some metalloporphyrins but it was restores the quenched fluorescence by other substrate such as anion. Therefore, the above system can be used as fluorescent sensor for determination of CN^-. In this study, *meso*-tetraphenylporphyrin cobalt (II) **(2)** was used as receptor unit for CN^- and **1** was used as fluorophore unit which does not require covalent bonding between the receptor and the fluorophore unit [1].

Methodology: Synthesis of **1** was achieved by using Rothemund and Alder-Longo reaction (Scheme 1). After refluxing for 30 minutes, the solution was cooled to room temperature. Then it was filtered and washed by hot water and methanol. The purple crystals of **1** would be obtained after air dried [2].



Scheme 1. Reaction of Rothemund and Alder-Longo reaction

Synthesis of **2** was achieved by Scheme 2 [3]. After refluxing the solution was cooled to room temperature in an ice bath and added distilled water and returned to the ice bath for an additional 5-10 min. The reaction mixture was filtered and washed with distilled water. **2** would be obtained after air dried.



Scheme 2. Reaction of synthesis of **2**

Study of the sensing properties: The fluorescent intensities of **1**/**2** system were determined with a spectrofluorometer (Variance Cary Eclipse) with excitation and emission wavelength of 416 and 650 nm respectively. A calibration curve for the sensing of CN^- was plotted and limit of quantitation was also calculated.

Results, Discussion and Conclusion:

*Characterization of meso-tetraphenylporphyrin (**1**) and meso-tetraphenylporphyrin cobalt (II) (**2**):* The IR spectrum of **1**(KBr: cm-1) was as followed: 3316(ν NH), 3052-3021(ν phenyl), 1575, 1558, 1541, 1507, 1489, 1472 and 1457 (plane skeletal vibration), 1219 (δ NH), 1000-723 (pyrrole vibration). For spectrum of **2** was as followed: 1600, 1475, 740 (aromatic characterization), 1348, 1504 and 1594 (C-C and C-N of benzene and porphyrin ring), 1004 (ν N-Co), 708, 752 and 802 (skeletal vibration of porphyrin ring).

^{1}H NMR of **1** (δ ppm, $CDCl_3$): 7.78 (3H×4, *m*, *p*-position of benzyl), 8.23 (2H×4, pyrrole), 8.84 (2H×4, *o*-position of benzyl).

^{1}H NMR of **2** (δ ppm, $CDCl_3$): 9.70 (1H×4, *p*-position of benzyl), 9.92 (2H×4, *m*-position of benzyl), 13.11 (2H×4, *o*-position of benzyl), 15.83 (2H×4, pyrrole).

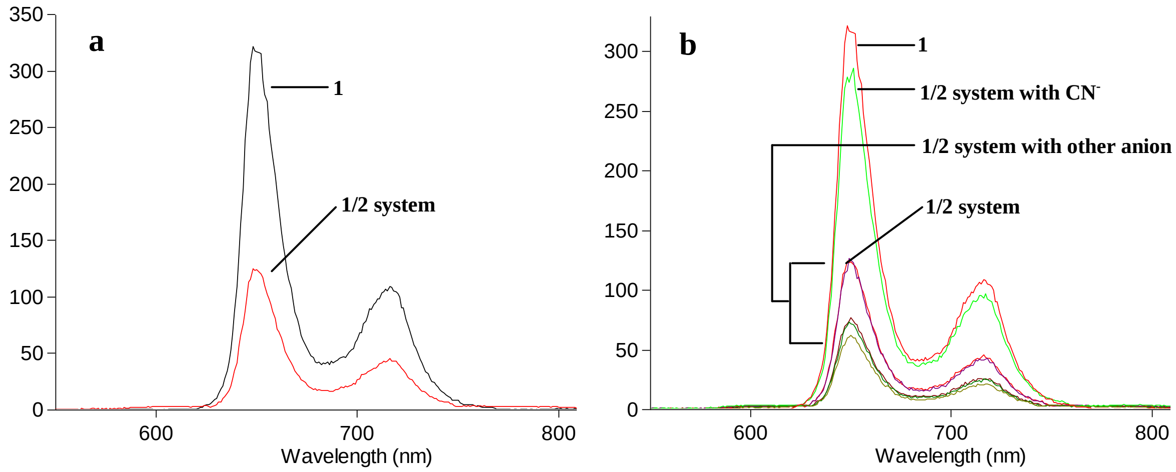


Figure 1. (a) Fluorescence spectra of **1** and **1**/**2** system, **(b)** Fluorescence spectra of **1** and **1**/**2** systems in which anions were added

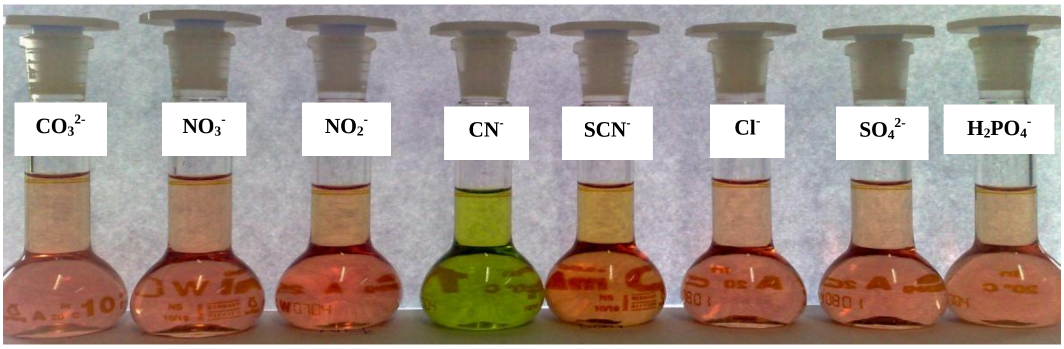


Figure 2. Color changes of **1**/**2** solution upon addition of different anions separately
[**1**] = 2.5 μM, [**2**] = 20 μM and [anion] = 7.5 mM

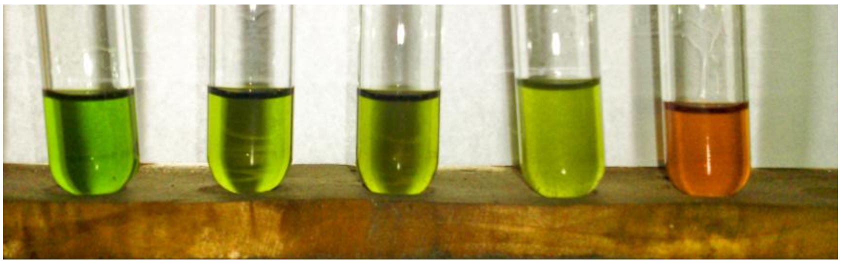


Figure 3. Color changes of **1/2** solution upon addition of different concentration of CN^- From left to right: 1 mM, 0.1 mM, 10 µM, 1µM and 0.1 µM

*The fluorescence behavior of **1**, **2** and **1/2** system:* Figure 1a shows that **1**, which did not have **2**, displayed a strong emission peak at 650 nm. When **2** was added into **1** solution, the fluorescence of **1** was significantly decreased. The quenched fluorescence was restored by adding anions into **1/2** system (Figure 1b). It was found that CN^- could remarkably restore fluorescence of the system among the anion ions examined. Moreover, the solution was initially pale red and change to green when CN^- was added. The result indicates that color change can be used for a naked-eye detection of CN^- (Figure 2). On the other hand, the green color could still be observed even at CN^- concentration of 1 µM and the shade of the green color also vary with the CN^- concentration (Figure 3).

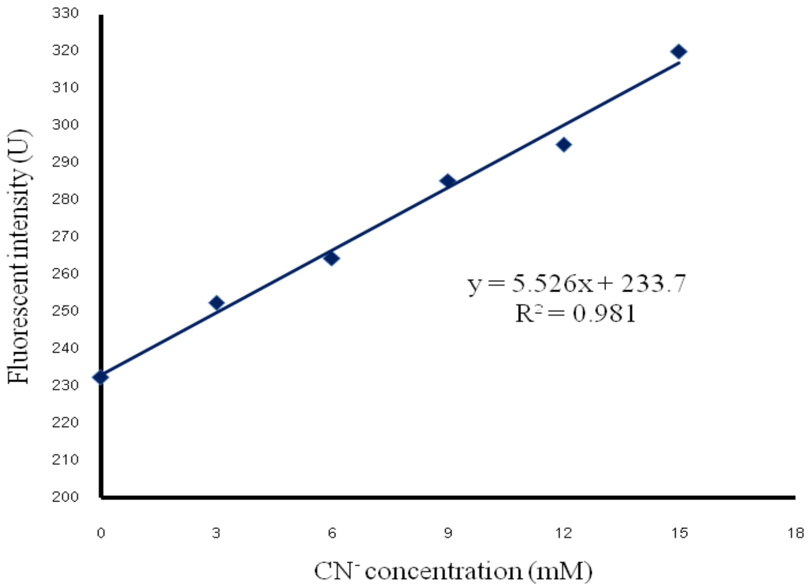


Figure 4. Calibration curve of determination of CN^- ion by **1/2** system

The performance of **1/2** system to recognize CN^- was also investigated. A great fluorescence enhancement accompanied with the increase of CN^- concentration. Linear calibration equation in the practically usable range was $y = 233.7 + 5.526x$ ($R^2 = 0.981$) where y is fluorescent intensity and x is the concentration of CN^- (Figure 4). The limit of quantitation of this practically system was 2.25 mM.

To test practical applicability of this system as a CN^- selective fluorescence sensor, competition experiments were carried out. The concentration of CN^- was fixed at 7.5 mM and then the changes of the fluorescence intensity were recorded before and after adding the interference into the solution. The results are shown in Table 1. Results from the table indicate that Cl^- and SCN^- are interfering ion for CN^- determination. SO_4^{2-}, NO_3^-, NO_2^- could also be the interfering ion for CN^- determination.

Table 1. Changes of the fluorescent intensity of **1**/**2** system before and after adding the interference into the solution

	$[CN^-]$	Interfering ion	Concentration	Fluorescent intensity (U)		
	(mM)		(mM)	Mean[a, b]	SD	%RSD
sample 1	7.5	–	–	278.56	3.08	1.1
sample 2	7.5	$H_2PO_4^-$	0.8	262.33	0.77	0.29
			7.5	287.41	0.54	0.18
			10.0	241.28	0.99	0.40
sample 3	7.5	SO_4^{2-}	0.8	282.77	4.36	1.54
			7.5	287.49	3.00	1.04
			10.0	217.14	2.06	0.95
sample 4	7.5	CO_3^{2-}	0.8	216.36	5.43	2.51
			7.5	271.49	3.79	1.39
			10.0	276.73	6.50	2.34
sample 5	7.5	NO_3^-	0.8	273.09	3.66	1.34
			7.5	280.16	2.62	0.93
			10.0	230.12	3.02	1.31
sample 6	7.5	Cl^-	0.8	349.76	1.02	0.29
			7.5	359.22	2.90	0.80
			10.0	246.22	5.14	2.08
sample 7	7.5	NO_2^-	0.8	276.70	6.40	2.31
			7.5	287.71	6.55	2.27
			10.0	264.48	1.00	0.37
sample 8	7.5	SCN^-	0.8	297.18	3.77	1.26
			7.5	306.14	3.11	1.01
			10.0	254.75	4.12	1.61

[a] average value form 3 replicates

[b] values presented in shade = interfere in the analysis of CN^- with 99% cofidence, values presented in unshade = not interfere in the analysis of CN^- with 99% confidence

In conclusion, we have synthesized *meso*-tetraphenylporphyrin (**1**) and *meso*-tetraphenylporphyrin cobalt (II) (**2**) as a fluorescent sensor for determination of CN^-. This system consists of two functional moieties: **1** performs as a fluorophore and **2** acts as a selected binding site for CN^- which does not require covalent bonding between the receptor and the fluorophore unit. The **1**/**2** system can practically use for determination of CN^-. However, we need further studies on the minimization effect from interfering ion in order to apply this system to determine CN^- in real sample.

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Keyword: Porphyrin, Fluorescent sensor, Cyanide ion

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