วารสารวิทยาศาสตร์บูรพา ปีที่ 26 (ฉบับที่ 1) มกราคม – เมษายน พ.ศ. 2564
BURAPHA SCIENCE JOURNAL Volume 26 (No.1) January – April 2021 บทความวิจัย

การใช้ไอออนโลหะในการควบคุมการตรวจวัดออกซาเลตแอนไอออนโดยใช้สารประกอบโคออร์ดิเนชันชนิดไดนิวเคลียร์ด้วยเทคนิคการถูกแทนที่ของอินดิเคเตอร์

Metal Ion Control the Selective Sensing of Oxalate Anion by Dinuclear Complexes under Indicator Displacement Strategy

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Received : 12 November 2020

Revised : 15 December 2020

Accepted : 19 December 2020

บทคัดย่อ

ได้ทำการสังเคราะห์และพิสูจน์โครงสร้างของสารประกอบโคออร์ดิเนชันชนิดไดนิวเคลียร์ของคอปเปอร์ (II) และสังกะสี (II) กับลิแกนด์ bis(dipicolylamine) ที่มี *para*-xylylene ทำหน้าที่เป็นสะพานเชื่อม bis(dipicolylamine) ทั้ง 2 หมู่ (**CuL1** และ **ZnL1**) หลังจากนั้นนำสารประกอบทั้งสองชนิดมาใช้เป็นรีเซ็บเตอร์สำหรับการตรวจวัดแอนไอออนโดยใช้อินดิเคเตอร์โบรโมไพโรแกลลอล เรด (bromopyrogallol red หรือ **BPG**) เป็นอินดิเคเตอร์ที่ใช้สำหรับตรวจวัดในตัวทำละลายผสม 80/20 (%v/v) ของอะซิโตรไรไตรล์และ HEPES ความเข้มข้น 10 mM ที่ pH 7.0 จากการศึกษาพบว่าเมื่อเติมแอนไอออนชนิดต่าง ๆ ลงไปยังสารละลายของเอนเซมเบิล [**CuL1•BPG**] และ [**ZnL1•BPG**] พบว่ามีเพียงเอนเซมบิล [**CuL1•BPG**] เท่านั้นที่สามารถตรวจสอบออกซาเลตแอนไอออนจากแอนไอออนชนิดต่าง ๆ ได้ โดยที่ออกซาเลตสามารถเปลี่ยนสีของสารละลายเอนเซมเบิลจากสีฟ้าอมม่วงไปเป็นสีม่วงอมชมพูของอินดิเคเตอร์ BPG ในรูปอิสระได้ จากผลการทดลองที่ได้จะเห็นได้ว่าชนิดของไอออนโลหะมีผลสำคัญต่อการตรวจวัดออกซาเลตแอนไอออน โดยที่การตรวจวัดออกซาเลตในงานวิจัยนี้สามารถตรวจวัดออกซาเลตในช่วงของความเข้มข้นที่ 20 – 50 µM (R^2 = 0.995) และมีค่าขีดจำกัดในการตรวจวัดด้วยตาเปล่าเท่ากับ 20 µM

คำสำคัญ : สารประกอบโคออร์ดิเนชันชนิดไดนิวเคลียร์ ; เทคนิคการถูกแทนที่ของอินดิเคเตอร์ ; ออกซาเลต ; การเห็นสีด้วยตาเปล่า ; เอนเซมเบิล

Abstract

Two dinuclear complexes of Cu(II) and Zn(II) with bis(dipicolylamine) linked by a *para*-xylylene bridge, **CuL1** and **ZnL1** have been synthesized and characterized. Both compounds are applied as metal-based indicator displacement assay (**IDA**) receptors for anions using bromopyrogallol red (**BPG**) as sensing indicators in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0. After addition of various anions to the solution of [**CuL1•BPG**] and [**ZnL1•BPG**] ensemble, the results showed that only [**CuL1•BPG**] could discriminate oxalate from other anions obviously resulting in the color change from blue-violet of ensemble to magenta color of free **BPG**. This result indicates that the nature of metal ion plays a crucial role to control the selective sensing of oxalate in this work. The quantitative detection of oxalate by [**CuL1•BPG**] ensemble was ranged from 20 – 50 μM, and a correlation coefficient (R^2) = 0.995. The detection limit was 20 μM by the naked eye.

Keywords : dinuclear complex ; indicator displacement assay ; oxalate ; naked eye ; ensemble

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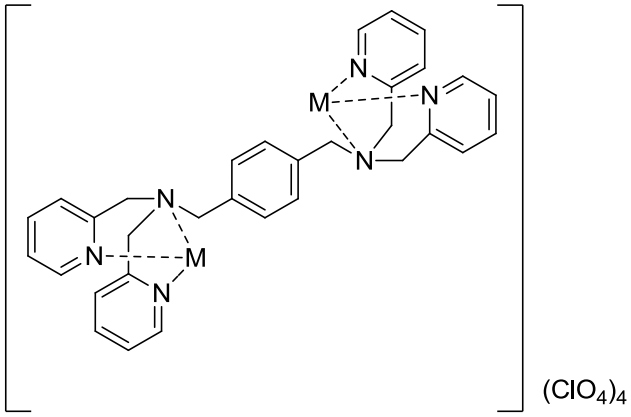
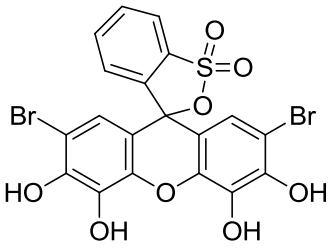
Introduction

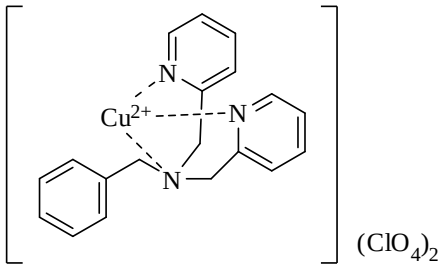
As one of the biological important anions, oxalate is naturally present in several foods and serves as a nutrient in the human body (Merusi *et al*., 2010). However, an excess consumption of oxalate is associated with the development of kidney stones (Ogawa & Miyazato, 2000), renal failure (Kasidas *et al.,* 1986 ; Marengo & Rose, 2008) and pancreatic insufficiency (Cartery *et al.,* 2011). Importantly, there is no enzyme in the human body to degrade oxalate. Therefore, oxalate is excreted by the kidney into the urine (Pundir *et al.*, 1998). Normal levels of oxalate for a healthy human are 10-30 mg/24 h (100-300 μM) in urine and 0.8 – 2.50 μM in plasma (Pundir *et al*., 1998). Hence, it is increasingly important to develop highly selective and sensitive oxalate. To date, several quantification analytical methods for oxalate detection mostly involved complicated and expensive equipment such as enzymatic methods (Kalra & Pundir, 2004), HPLC (Honow *et al*., 2002), capillary electrophoresis (Munoz * Lopez, 2010 ; Noblitt *et al.*, 2009), gas chromatography (Li *et al.*, 2008) and electrochemical methods (Rodriguez *et al.*, 2012) . Based on the current researches of anions detection, chemosensors for oxalate are more active in the field of detection because of their low price and visually detectable (Suksai & Tuntulani, 2003 ; Curriel *et al.,* 2015). For example, Bhattacharya and co-workers have employed rhodamine complex with Cu(II) for quantification of oxalate in agricultural crops, drinking and human urine (Dey *et al.*, 2018). Dungchai and co-workers have constructed paper-based colorimetric device for point-of-care monitoring oxalate in artificial urine sample (Worramongkona *et al.*, 2018). Indicator displacement assay (IDAs) is an alternative strategy for chemosensors due to its conveniences for constructions the visual observation systems. The recognition unit and sensory unit interact by non-covalent interactions (Wiskur et al., 2001 ; Lavigne & Anslyn, 2006 ; Nguyen & Anslyn, 2006). Recently, colorimetric detection of oxalate by IDAs has been reported (Tang & Liu, 2010 ; Su *et al*., 2010 ; Hu & Feng, 2012 ; Rhaman *et al*., 2014 ; Tang *et al*., 2014 ; Inoue *et al*., 2018). Herein, in the continuation of our ongoing research on the construction of dinuclear complex of copper(II) or Zn(II) as meal based IDA receptors for anion (Watchasit *et al*., 2010 ; Watchasit *et al*., 2014), we reported the synthesis of homodinuclear complexes of Cu(II) and Zn(II) with bis(dipicolylamine) linked by a *para*-xylylene bridge, **CuL1** and **ZnL1**, and investigated the sensing abilities of those two complexes as metal-based IDA receptors for oxalate and using bromopyrogallol red (**BPG**) as the sensing indicator in 80% acetronitrile aqueous solution buffered with 10 mM HEPES pH 7.0. We anticipated that the nature of meal ion center will lead to the discriminate sensing for oxalate from other anions and also interested dicarboxylates anions. Interestingly, we found that the dinuclear **CuL1** complex is a suitable receptor for indicator displacement assay of oxalate. Moreover, the anion sensing abilities of the mononuclear complex **CuL2** have also studied compared to the dinuclear complex **CuL1**. The structures of **CuL1**, **ZnL1**, **CuL2** and **BPG** are shown in Figure 1.



ML1 (where M = Cu^{2+} or Zn^{2+})



Bromopyrogallol Red (BPG)



CuL2

Figure 1 Structures of CuL1, ZnL1, CuL2 and BPG

Methods

1. Materials and methods

All chemicals were at least of analytical grade and used without further purification. Sterile water for injection was obtained from General Hospital Products Public Co., Ltd, (PathumThani, Thailand). All UV-vis absorption spectra were recorded using an Agilent 8453 UV-vis spectrophotometer. All complexes and indicator solutions were freshly prepared immediately before UV-vis experiments. ^{1}H- and ^{13}C-NMR spectra were carried out using Bruker AVANCE III HD 400 MHz Ultra Shield spectrometer. Dipicolylamine (**DPA**) was synthesized according to the previously published procedure (Watchasit *et al.*, 2010).

2. Synthesis of L1

The mixtures of 1,4-bis(bromomethyl)benzene (3.25 g, 12.32 mmol), bis(pyridin-2-ylmethyl)amine (5.86 g, 29.45 mmol), Cs_2CO_3 (9.58 g, 29.40 mmol) and KI (3.06 g, 18.43 mmol) in CH_3CN (150 mL) were dissolved in dry CH_3CN. The reaction mixture was reflux under nitrogen atmosphere for 24 h. The dark precipitate was filtered. The filtrate was removed solvent under vacuum. The residual was redissolved in CH_2Cl_2 (100 mL) and washed with water (3 × 300 mL). The organic extracts were dried with anhydrous Na_2SO_4. After solvent removal, a dark oil of **L1** was obtained and then purified by column chromatography (silica gel, MeOH : CH_2Cl_2 = 2:8 v/v). The light yellow solid of **L1** was obtained (0.23 g, 2.36%) after recrystallization in acetone and water.

^{1}H-NMR (400 MHz, CD_3CN, ppm): δ 8.47-8.45 (m, 4H, Ar*H*), 7.73-7.69 (m, 4H, Ar*H*), 7.61 (s, 2H, Ar*H*), 7.59 (s, 2H, Ar*H*), 7.37 (s, 4H, Ar*H*), 7.20-7.16 (m, 4H, Ar*H*), 3.73 (s, 8H, -CH_2-), 3.63 (s, 4H, -CH_2-).

^{13}C-NMR (100 MHz, CD_3CN, ppm): δ 159.73, 148.78, 137.99, 136.43, 128.76, 122.77, 100.02, 59.66, 57.84.

HRMS ESI positive mode ; 501.2785 [**L1**+H]$^+$, 523.2606 [**L1**+Na]$^+$.

3. Synthesis of CuL1

The water solution of $Cu(ClO_4)_2.6H_2O$ (0.74 g, 2 mmol) was gradually added to acetonitrile solution suspension of **L1** (0.2 g, 0.40 mmol) and the color of solution changed to blue immediately. Then, the mixture was heated for 10 min. After cooling to room temperature, the blue solid precipitate was filtered and washed with CH_2Cl_2 to obtain **CuL1** (0.28 g, 68 % yield). HRMS ESI positive mode ; 924.9718 [**CuL1**+ $3ClO_4^-$]$^+$.

4. Synthesis of ZnL1

The ethanol solution of $Zn(ClO_4)_2.6H_2O$ (0.74 mg, 2 mmol) was added to the ethanol solution suspension of **L1** (0.2 g, 0.40 mmol) and then ether and hexane were added into the solution mixture, respectively. The white solid precipitate was filtered and washed with CH_2Cl_2 to obtain **ZnL1** (0.15 g, 40 % yield).

^{1}H-NMR (400 MHz, CD_3CN, ppm): δ 8.80 (d, *J* = 4.8 Hz, 4H, Ar*H*), 8.24-8.19 (m, 4H, Ar*H*), 7.77-7.69 (m, 8H, Ar*H*), 7.37(s, 4H, Ar*H*), 4.32(d, *J* = 16.0 Hz, 4H, -CH_2-), 3.90 (t, *J* = 16.4 Hz, 8H, -CH_2-).

^{13}C-NMR (100 MHz, CD_3CN, ppm): δ 154.49, 148.11, 141.99, 132.05, 131.91, 125.46, 125.27, 55.64, 55.02.

HRMS ESI positive mode ; 928.9745 [**ZnL1**+ $3ClO_4^-$]$^+$.

5. Synthesis of L2

The mixtures of (bromomethyl)benzene (1.86 g, 10.88 mmol), bis(pyridin-2-ylmethyl)amine (2.60 g, 13.07 mmol), Cs_2CO_3 (4.30 g, 13.20 mmol) and KI (2.74 g, 16.50 mmol) were dissolved in CH_3CN (150 mL). The reaction mixture was reflux under nitrogen atmosphere for 24 h. The dark precipitate was filtered. The filtrate was removed solvent under vacuum. The residual was redissolved in CH_2Cl_2 (100 mL) and washed with water (3 × 300 mL). The organic extracts were dried with anhydrous Na_2SO_4. After solvent removal, a dark oil of **L2** was obtained and then

purified by column chromatography (silica gel, MeOH : CH_2Cl_2 = 1:9 v/v). The light yellow liquid of **L2** was obtained (1.34 g, 42.34%).

^{1}H-NMR (400 MHz, CD_3CN, ppm): δ 8.53 (t, *J* = 11.6 Hz, 2H, Ar*H*), 7.70-7.60 (m, 4H, Ar*H*), 7.42 (d, *J* = 7.2 Hz 2H, Ar*H*), 7.35- 7.13 60 (m, 5H, Ar*H*), 3.82 (d, *J* = 3.6 Hz, 4H, -CH_2-), 3.70 (s, 4H, -CH_2-).

^{13}C-NMR (100 MHz, CD_3CN, ppm): 159.69, 148.87, 138.90, 136.47, 128.83, 128.30, 127.06, 122.80, 121.97, 60.02, 58.52. HRMS ESI positive mode ; 290.1753 [**L2**+H^+].

6. Synthesis of CuL2

The ethanolic solution of $Cu(ClO_4)_2.6H_2O$ (1.28 mg, 3.46 mmol) 10 mL was added to the ethanolic solution of **L2** (0.2 mg, 6.91 mmol) 10 mL. The reaction mixture was stirred at room temperature for 3 hours. Then, diethyl ether 100 mL was added to the blue solution and, stirred at room temperature for 30 min. The blue solid precipitate was filtered and washed with CH_2Cl_2 to obtain mononuclear **CuL2**. (0.24 g, 63.02%).

HRMS ESI positive mode ; 451.0449 [**CuL2** + $3ClO_4^-$]$^+$

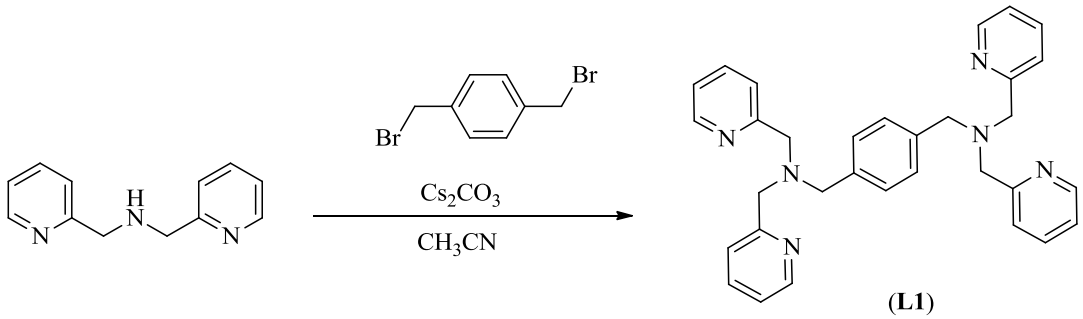
7. Screening for selective anion sensing

A stock solution of **CuL1**, **ZnL1** and **CuL2** (20 μM) and **BPG** (400 μM) was prepared in 20% (v/v) water/acetonitrile solutions buffered at pH 7.0 with HEPES. A solution of **BPG** 400 μM (0.1 mL) in 20% (v/v) water/acetonitrile solutions buffered at pH 7.0 with HEPES was added into a solution of each complex of **CuL1**, **ZnL1** and **CuL2** 20 μM (2 mL) in the same solvent system. Then, 10 equivalents of interested anions (1 mM) was then added to the as-prepared ensemble. The resulting mixtures were allowed to stand still for 5 min and subjected to UV-vis spectroscopic measurements. Photographs were taken by a digital camera (Canon EOS 7D with Tamron 17-50 mm F2.8 lens).

Results

1. Synthesis of L1, CuL1 and ZnL1

Ligand **L1** was synthesized in one step reaction as shown in Scheme 1. The dipicolylamine was reacted with 1,4-bis(bromomethyl)benzene in refluxing acetronitrile using Cs_2CO_3 as base to yield **L1** in 30% yield. The HRMS spectrum of **L1** showed the parent peak at m/z at 501.2784 amu and 523.2066 amu assigned to the molecular ion of [**L1** + H]$^+$ and [**L1**+ Na]$^+$, respectively (Figure S7 , ESI).



Scheme 1. Synthesis procedure of **L1**

Addition of $Cu(ClO_4)_2$ or $Zn(ClO_4)_2$ to methanolic solutions of **L1** gave **CuL1** and **ZnL1** in 68% and 40%, respectively. The HRMS spectrum of green solid of **CuL1** showed the parent peak at m/z 924.9718 amu which is assigned to the molecular ion of the mononuclear complex of $[\mathbf{CuL1}+3(ClO_4)]^+$ species (Figure S8, ESI). For **ZnL1**, the parent peak at m/z 928.9745 amu corresponding to the molecular ion of the dinuclear complex $[\mathbf{ZnL1}+3(ClO_4)]^+$ was observed (Figure S9, ESI). All ^1H and ^{13}C-NMR of **L1**, **L2** and **ZnL1** are shown in Figure S1-S6 in ESI.

2. Sensing abilities studies of CuL1 and ZnL1 with BPG ensemble towards anions

The addition of **CuL1** or **ZnL1** to **BPG** solution in 80% acetronitrile aqueous solution buffered with 10 mM HEPES pH 7.0 resulting in the color change from magenta color of free **BPG** to blue-violet color of ensemble suggested that those two complexes could form the colorimetric ensemble with **BPG**. Then, those two ensembles were treated with several interested anions in the same solvent system. As is evident from inspection of Figure 2, only ensemble formation between **BPG** and **CuL1** showed the highly selective sensing toward oxalate anion in which only oxalate could change the color of the ensemble solution from blue-violet color to magenta color, Figure 2(a). In the case of **ZnL1** ensemble with **BPG**, it was not only selective to oxalate anion but also several anions could turn the blue-violet color of ensemble to magenta color of free **BPG** for example PPi, TPP, AMP and ATP. This result might be expected that the binding affinity of **BPG** to **ZnL1** was lower than that of **CuL1**.

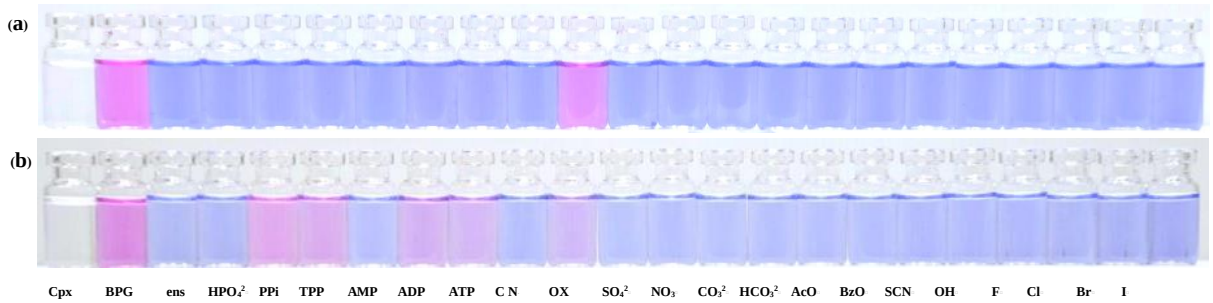


Figure 2 Color changes of ensemble (a) CuL1 (20 μM) and (b) ZnL1 (20 μM) with indicator BPG (20 μM) after addition of various anions (12.5 equivalents). All experiments have been carried out in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0.

Next, the sensing abilities of [CuL1•BPG] and [ZnL1•BPG] have further studies by UV-visible spectrophotometry. Figure 3(a) showed that upon addition of various anions (12.5 equivalents) to the [CuL1•BPG] ensemble solutions, only oxalate showed the bathochromic shift from concomitantly to the color of solution change from blue-violet to magenta of the unbound dye, whereas no noticeable absorption changes were observed upon addition of other anions. In contrast to [ZnL1•BPG] ensemble, it was not only selective to oxalate but also other anions could alter the spectrum. These results suggested that [CuL1•BPG] possessed a high selectivity towards oxalate anion over other common anions.

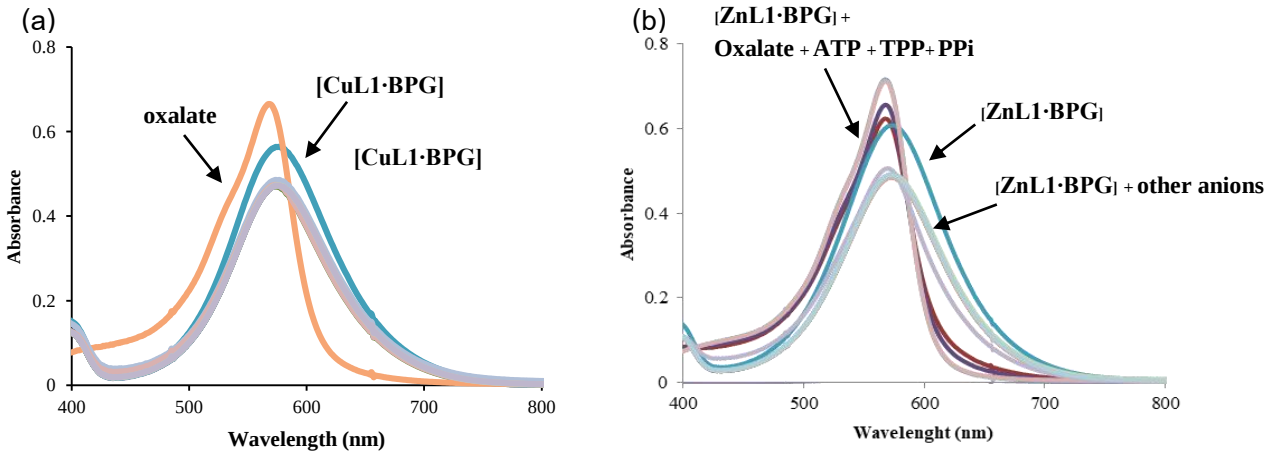


Figure 3 UV-vis spectra obtained by addition of various anions (10 equiv. of sodium salts) to an ensemble solution of (a) [CuL1•BPG] and (b) [ZnL1•BPG] (20 μM) in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0.

3. Studies of ensemble formation constants between CuL1 and ZnL1 with BPG by UV-vis spectrophotometry

The ensemble formation constants of **BPG** with **CuL1** and **ZnL1** were determined by UV-vis titration experiments. The magenta color of free **BPG** was titrated with increasing amounts of each **CuL1** and **ZnL1** complex in 80% acetronitrile aqueous solution buffered with 10 mM HEPES pH 7.0. The absorption spectrum of free **BPG** exhibited an absorption band centered at 567 nm. Upon addition of **CuL1** or **ZnL1** to **BPG** solution, the absorbance at 567 nm decreased concomitantly to the bathochromic shift to 574 nm, with a significant visible color change from magenta to blue-violet color, Figure 4. The absorption at 574 nm reached saturation when 1.4 equivalent of **CuL1** or **ZnL1** was added. The stoichiometric ratios between **BPG** with **CuL1** or **ZnL1** were evaluated by Job's plot analysis. The results suggested that the tertiary complexes between **BPG** with **CuL1** or **ZnL1** were formed with a 1:1 stoichiometry (Figure S10, ESI). Moreover, the ensemble formation constants (log β) of two ensembles have calculated using SPECFIT computer program (Binstead *et al*., 2000 ; Gampp *et al*., 1986 ; Gampp *et al*., 1985) and found to be 5.98 ± 0.26 and 5.20 ± 0.41 for [**CuL1•BPG**] and [**ZnL1•BPG**], respectively. It should be reminded that the ensemble formation constant of [**CuL1•BPG**] was higher than that of [**ZnL1•BPG**] as we expected and agreed well with Irving-Williams series.

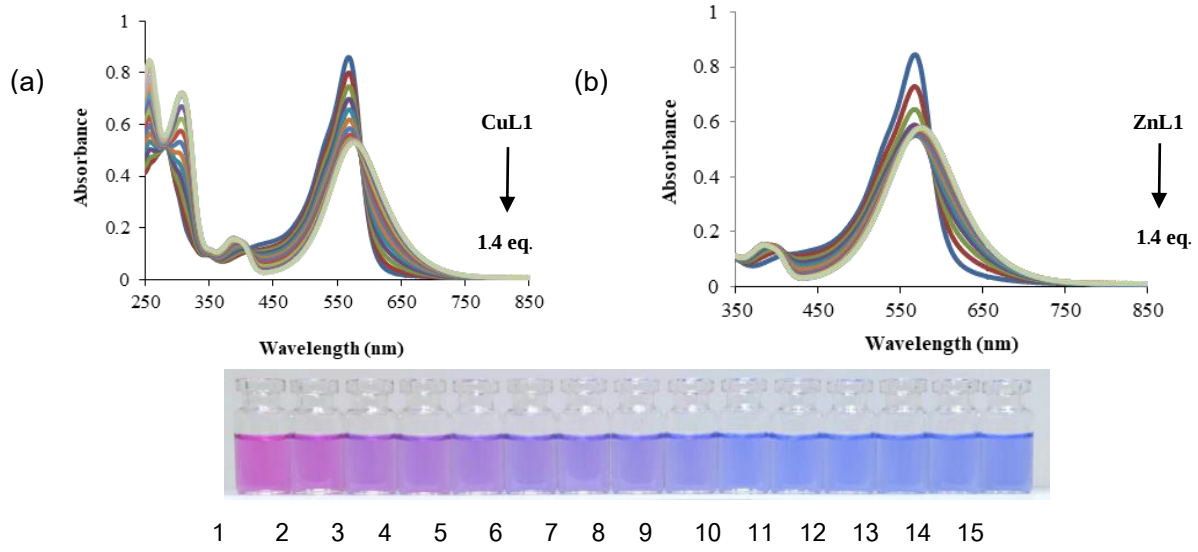


Figure 4 UV/vis spectra obtained by addition of (a) **CuL1** (400 μM) and (b) **ZnL1** (400 μM) to ensemble solution of **BPG** (20 μM) in 80/20 (%v/v) acetronitrile/water solution with 10 mM HEPES buffered at pH 7.0. (c) The color changes of **BPG** solution as a function of equivalents of **CuL1** in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0 : **(1) BPG, (2)** 0.1 eq., **(3)** 0.2 eq., **(4)** 0.3 eq., **(5)** 0.4 eq., **(6)** 0.5 eq., **(7)** 0.6 eq., **(8)** 0.7 eq., **(9)** 0.8 eq., **(10)** 0.9 eq., **(11)** 1.0 eq., **(12)** 1.1 eq., **(13)** 1.2 eq., **(14)** 1.3 eq. and **(15)** 1.4 eq. of **CuL1**.

4. Sensing studies of oxalate by [CuL1•BPG] ensemble under IDAs

The displacement of bound **BPG** by oxalate was studied by UV-vis spectrometry, Figure 5. Increasing oxalate concentration to an ensemble solution of [**CuL1•BPG**] caused an absorbance increase around 567 nm and an absorbance decrease around 574 nm (hypsochromic shift), with a color change from magenta to blue-violet, revealing that the **BPG** indicator was displaced from the cleft of **CuL1** by the oxalate. The UV-vis spectrum at 567 nm was completely saturated at 12.5 equivalents of oxalate. The apparent competitive binding constant (log β) of [**CuL1**•2(oxalate)] was found to be 6.25 ± 0.38 using SPECFIT computer program. In addition, the formation of 1:2 complex species of [**CuL1**•2(oxalate)] could be confirm by HRMS that shown in Figure 5(b). The cluster of m/z at 928.9898 amu corresponding to the adduct species of [**CuL1** + 2(oxalate) + ClO_4^- + Na^+ + H^+]$^+$ could be clearly observed. According to HRMS result, we expected that each oxalate anion acts as bidentate ligand coordinate to each Cu^{2+} ions in **CuL1** possessing the 1:2 complex cation of **CuL1**:2(oxalate). The proposed structure of the ternary complex of **CuL1** and oxalate anion was shown in Figure 5(b).

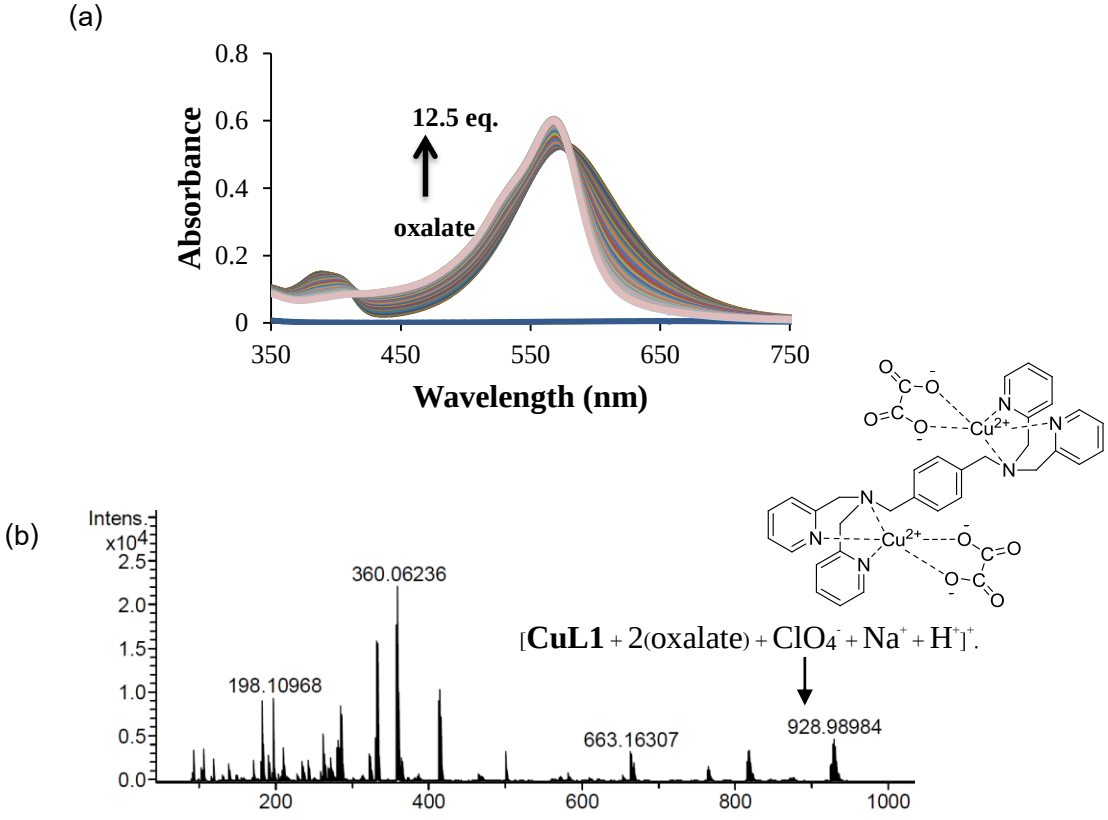
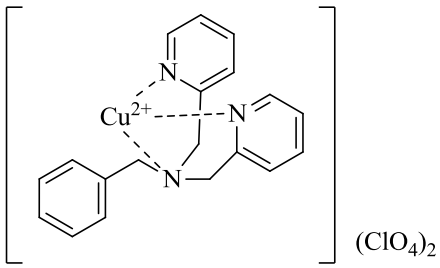


Figure 5 (a) UV/vis spectra obtained by addition of oxalate (1 mM) to ensemble solution of [**CuL•BPG**] (20 μM) in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0 and (b) HRMS spectrum of [**CuL1** + 2(oxalate) + ClO_4^- + Na^+ + H^+]$^+$ and it's proposed structured.

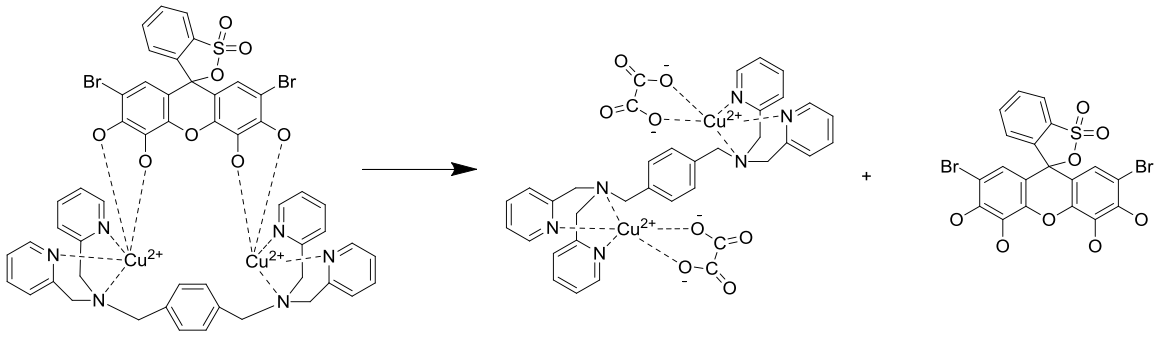
According to mass spectrometry results, we concerned that the mononuclear complex **CuL2** can be exploited as the M-IDA receptor for oxalate instead of **CuL1**. Therefore, we decided to synthesize ligand **L2** and **CuL2** complex and also investigated the sensing abilities of [**CuL2•BPG**] ensemble towards the same series of anions and also in the same solvent system.



CuL2

Figure 6 Color changes of ensemble [**CuL2•BPG**] 20 μM after addition of various anions (5.0 equivalents). All experiments have been carried out in 80/20 (%v/v) acetronitrile/water solution buffered with 10 mM HEPES at pH 7.0.

Obviously, Figure 6 showed that the [**CuL2•BPG**] ensemble could not be able to discriminate oxalate anion selectively because other anions, especially phosphate containing anions could change the purple color of ensemble to magenta color of free **BPG**. It could be expected that in [**CuL2•BPG**] structure, **BPG** might be coordinated to Cu^{2+} ion in **CuL2** complex in bidentate fashion. Therefore, several bidentate and polydentate ligands can replace the bound **BPG** from ensemble easily. Contrast to [**CuL1•BPG**] ensemble, **BPG** acts as tetradentate ligand and coordinates to two Cu^{2+} ion in **CuL1** more tightly. Therefore, the naked-eye sensing mechanism of oxalate by [**CuL1•BPG**] ensemble under indicator displacement assay are shown in Scheme 2.



Scheme 2. Sensing mechanism of oxalate under IDAs by [**CuL1·BPG**] ensemble.

Discussion

To investigate sensing ability, [**CuL1•BPG**] ensemble can be utilized as a selective naked-eye sensor for oxalate against other anions, the competition experiments with oxalate and the other anions were conducted in 80% acetronitrile aqueous solution buffered with 10 mM HEPES pH 7.0, as shown in Figure 7. Compared to [**CuL1•BPG**] ensemble containing oxalate, no obvious changes in the absorbance at 574 nm were observed with the other anions. These results strongly recommend that the coexistence of other anions could not interfere the sensing of oxalate under displacement strategy. Therefore, [**CuL1•BPG**] ensemble exhibits the highest selectivity toward oxalate. The absorbance at 567 nm was plotted as a function of oxalate concentration and showed a good linear relationship between absorbance change and oxalate concentration in the range of 20 - 50 μM of oxalate with a linearly dependent coefficient, R^2 = 0.995. The detection limit of oxalate by naked eye was found at 20 μM.

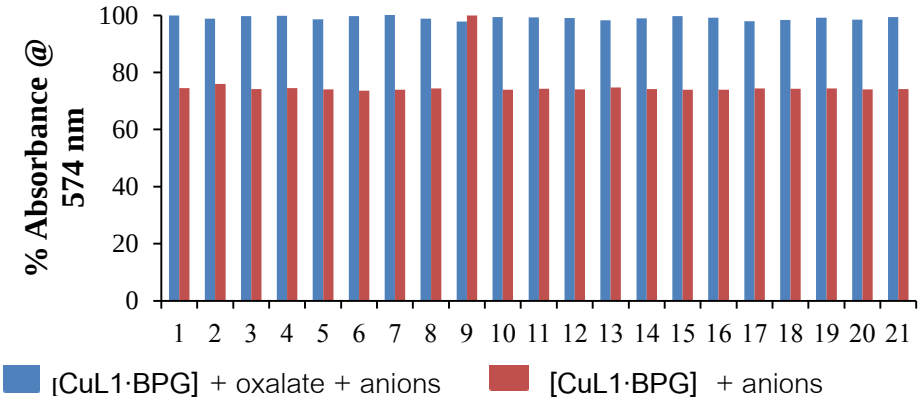


Figure 7 Sensing of oxalate by [**CuL1·BPG**] ensemble in the presence of competitive anions (12.5 equiv.) in 80/20 (v/v) MeCN/H_2O solution buffered with 10 mM HEPES at pH 7. (1) = buffer, (2) = Pi (3) = PPi, (4) = TPP, (5) = AMP, (6) = ADP, (7) = ATP, (8) = CN^-, (9) = ox, (10) = SO_4^{2-}, (11) = NO^{3-}, (12) = CO_3^{2-}, (13) = HCO_3^-, (14) = AcO^-, (15) = BzO^-, (16) = SCN^-, (17) = OH^-, (18) = F^-, (29) = Cl^-, (20) = Br^- and (21) = I^-.

Conclusion

In conclusion, we successfully synthesized two dinuclear complexes of Cu(II) and Zn(II) bearing dipicolylamine containing *p*-xylylene scaffold, **CuL1** and **ZnL1**, respectively. Both compounds have been investigated as IDA receptors for anions by using **BPG** as sensing indicator. We have found that only [**CuL1•BPG**] ensemble could discriminate oxalate anion from other common anions and dicarboxylate anions because oxalate anion could displace the bound **BPG** from ensemble cavity. In contrast to [**ZnL1•BPG**], several anions could replace the bound **BPG** from the ensemble structure. It could be implied that the binding constant of **BPG** with **CuL1** is stronger than that of **ZnL1**. Therefore, in this work the nature of metal ion plays a crucial role to control the selective sensing for oxalate anion over other anions.

Supporting Information

Additional ^{1}H and ^{13}C NMR spectra of **L1**, **ZnL1** and **L2**. Please contact corresponding author for detail of supporting information.

Acknowledgement

This work was partially supported by the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education. The authors acknowledge the technical support provided by Faculty of Science, Burapha Unversity, Thailand.

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