Symmetrical Azine Colorimetric and Fluorometric Turn-Off Chemosensor for Formaldehyde Detection
(Kolorimetri Simetri Azina dan Fluorometri Mematikan Kemoderia untuk Pengesanan Formaldehid)

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ABSTRACT

Azine derivative 6,6’-((1E,1’E)-hydrazine-1,2-diylidenebis(methanyli-lidene))bis(3,4-dimethoxy-aniline) CHA has been synthesized by condensation of 6-nitroveratraldehyde with malononitrile followed by reduction using 10% Pd/C and hydrazine hydrate. In the presence of formaldehyde, CHA chemosensor in ethanol showed a yellow-to-red color change observable by the naked eyes and ‘turn off’ type fluorescence quenching in ethanol. This phenomenon was confirmed by UV-Vis, fluorescence and $^1$H NMR spectroscopy. The CHA spectra in ethanol was shifted from 412 to 509 nm after addition of formaldehyde. Fluorescence intensity of CHA gradually decreased with the increase of formaldehyde concentrations. Furthermore, paper strips loaded-CHA were fabricated and served to detect formaldehyde qualitatively. The detection limit of the CHA probe to formaldehyde is 0.2 M.

Keywords: Azine; chemosensor; colorimetric; fluorometric; formaldehyde; turn off

ABSTRAK

Terbitan azina CHA 6,6’-((1E,1’E)-hidrazina-1,2-diidienebis(metanili-liden))bis(3,4-dimetoksi-anilin) telah disintesis dengan pemeluwapan 6-nitroveratraldehid dengan malononitril diikuti dengan pengurangan menggunakan 10% Pd/C dan hidrat hidrazina. Dengan kehadiran formaldehid, kemoderia CHA dalam etanol menunjukkan perubahan warna daripada kuning kepada merah yang boleh diperhatikan oleh mata kasar dan ‘mematikan’ jenis pemelindapan kependarfluoran dalam etanol. Fenomena ini disahkan oleh UV-vis, kependarfluoran dan spektroskopi $^1$H NMR. Spektrum CHA dalam etanol telah beralih daripada 412 kepada 509 nm selepas penambahan formaldehid. Keamatan kependarfluoran CHA secara bersans-ansur menurun dengan peningkatan kepekatan formaldehid. Tambahhan pula, jalur kertas yang dimuatkan-CHA telah direka dan digunakan untuk mengesan formaldehid secara kualitatif. Had pengesanan prob CHA kepada formaldehid adalah 0.2 M.

Kata kunci: Azina; fluorometrik; formaldehid; kemoderia; kolorimetri; mematikan

INTRODUCTION

Formaldehyde is usually found in resins used in manufacturing of wood products, building materials, household products, fertilizers, and pesticides. In addition, formaldehyde is also used as a food preservative illegally, on fish and meat products, wet noodles, and soybean curds. On the other hand, formaldehyde has serious effects on human health such as irritation of the skin, eyes, nose, throat and causes DNA damage (Merk 1998). Moreover, a high concentration of formaldehyde exposure has been reported to cause cancer (Cogliano et al. 2005). For these reasons, it is necessary to develop a simple and cost-effective formaldehyde chemosensor.

Research regarding formaldehyde detection methods include using chromatography, spectrophotometry, and enzymatic methods. Li et al. (2007) and Yeh et al. (2013) determined the formaldehyde level in a sample using HPLC and GC-MS. This method has involved longer analysis time, complicated tools, and therefore cannot be used for routine analysis. Korpan et al. (1993) and Nikitina et al. (2007) have designed biosensors to detect formaldehyde enzymatically. However, it has a low potential because it involves a lot of interference and depended on the environmental conditions (pH and temperature), where handling is very complicated. Dar et al. (2013) used Thin Layer Chromatography (TLC) method for formaldehyde determination but this method was unable to measure formaldehyde of samples in high concentration. Spectrophotometric methods in detection of formaldehyde are divided into colorimetric (Mohr 2003; Wei et al. 2019) and fluorometric (Bi et al. 2018; Dong et al. 2016; He et al. 2016; Hidayah et al. 2019; Liu et al. 2015; Ovianto et al. 2017; Roth et al. 2015; Song et al. 2012; Wu et al. 2018; Xu et al. 2016; Zhang et al. 2018; Zhou et al. 2018, 2015). The method has advantages over other methods, namely simpler, easier to handle, cheaper, higher in sensitivity and can be used for routine analysis (Chen et al. 2015; Indang et al. 2009; Li et al. 2007).

Azine derivatives have been reported as colorimetric and fluorometric sensors for cation, anion, and netral compound (Caballero et al. 2005; Kaushik et al. 2018; Li et al. 2011; Martinez et al. 2005; Pei et al. 2018; Peng et al. 2014; Suresh et al. 2010). Many fluorescent sensors...
for the detection of formaldehyde have been developed, but majority of the publications discuss the ‘turn on’ type probes (He et al. 2016; Liu et al. 2015; Song et al. 2012; Xu et al. 2016; Zhang et al. 2018; Zhou et al. 2015). In this work, we report a dual-mode chemosensor for formaldehyde detection from symmetrical azine derivative CHA. The probe can be synthesized from condensation of 6-nitroveratraldehyde and malononitrile followed by one-step reduction of CHA-1 to produce the target probe of CHA (Figure 1). The chemosensor CHA can be used for formaldehyde detection through colorimetric (produces a distinct color change from yellow to red) and ‘turn off’ fluorescent quenching mechanism in ethanol.

**MATERIALS AND EQUIPMENTS**

Materials used for synthesis were 6-nitroveratraldehyde (C₉H₅NO₂), malononitrile (CH₂(CN)₂), 10% Pd/C, hydrazine hydrate 80%, and distilled water. Solvent used for UV-Vis and fluorescence spectra measurement was absolute ethanol. These chemicals were pro analytic reagents from Merck, except for distilled water.

Equipment used for the synthesis was a set reflux, hot plate magnetic stirrer, and laboratory glassware. Instruments employed for characterization of synthesis product were melting point (Electrothermal 9100), FT-IR spectrophotometer (Shimadzu Prestige 21), Gas Chromatography-Mass Spectrometry (Shimadzu QP-2010S), and NMR spectrometer (JEOL JNM-ECZ500R). For UV-Visible and fluorescence measurements, spectrophotometer UV-Vis (Shimadzu UV-1800) and spectrofluorophotometer (Shimadzu RF-6000) were used.

**METHODS**

**SYNTHESIS OF 2-(4,5-DIMETHOXY-2-NITROBENZYLIDINE) MALONONITRILE (CHA-1)**

The synthesis of 2-(4,5-dimethoxy-2-nitrobenzylidene) malononitrile CHA-1 was accomplished using a modified method from Li et al. (2015). A mixture of malononitrile (2 mmol) and 6-nitroveratraldehyde (2 mmol) in water was refluxed for 24 h and the progress of the reaction was monitored by TLC. After the completion of the reaction, the solid product was filtered, washed with water and stored in desiccator. The 2-(4,5-dimethoxy-2-nitrobenzylidene) malononitrile was obtained by recrystallization from ethanol and water as greenish-needle-shaped solid (0.38 g, 60.81%, mp 145-146°C). FT-IR (KBr) v (cm⁻¹): 1064 and 1180 (C=O), 1604 (C=N), 1519 and 1327 (NO₂); ¹H NMR (500 MHz, CDCl₃) δ (ppm): 4.05 (s, 6H), 7.26 (s, 1H), 7.80 (s, 1H), 8.46 (s, 1H); MS (EI) for C₁₁H₂₂N₂O₄ m/z: 259.0 (M⁺).

**SYNTHESIS OF 6,6’-(1E,1’E)-HYDRAZINE-1,2-DIYLIDENEBIS(METHANYLYLIDENE)BIS(3,4-DIMETHOXYANILINE) (CHA)**

The synthesis of 6,6’-(1E,1’E)-hydrazine-1,2-diylidenebis(methanylylidene)bis(3,4-dimethoxyaniline) (CHA) was accomplished using a modified Tamami and Yeganeh (2001) method. To a solution of CHA-1 in ethanol (0.5 mmol) was added 10% Pd/C (0.04 g). The mixture was stirred and warmed to about 50°C, whereupon 80% hydrazine hydrate 0.4 mL in 1 mL of ethanol was added slowly using dropping funnel. After the addition was completed, the mixture was refluxed for 2 h at 78°C (the reaction was monitored by TLC) and filtered while it was still hot. The crude product as solid was obtained from evaporation of the solvent. The solid was recrystallized from water and ethanol as yellow solid (0.11 g, 44%, mp 260-261°C). FT-IR (KBr) v (cm⁻¹): 1141 and 1188 (C-O-C), 1242 and 1273 (C-N), 1604 (C=O), 3371 and 3441 (NH). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 3.83 and 3.88 (s, 6H), 6.07 (s, 2H), 6.24 (s, 1H), 6.73 (s, 1H), 8.62 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 55.98, 56.82, 99.32, 108.09, 115.99, 1327 (NO₂), 1604 (C=O), 1519 and 1327 (NO₂); MS (EI) for C₁₆H₂₅N₄O₄ m/z: 358 (M⁺).

**GENERAL PROCEDURE FOR UV-VISIBLE AND FLUORESCENCE SPECTRA MEASUREMENT**

Generally, the procedure to generate the UV-Vis and fluorescence spectra were the same with the previous work by Hidayah et al. (2019). The solutions of chemosensor CHA was prepared by dissolving CHA solid in ethanol until it dissolves completely. The 37% of formaldehyde in water (saturated solution=13.3 M) were used to make the final concentration of 1×10⁻² M. Formaldehyde concentration of 0, 1, 3, 5, 7, 9, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200×10⁻² M was added into CHA chemosensor ethanol solution and then the UV-visible and fluorescence spectra were recorded.

**FIGURE 1. Synthetic route of compound CHA**
APPLICATION OF CHA AS PAPER STRIP

The paper strip for qualitative testing was prepared by immersing the filter papers (Whatman no. 42) in the CHA ethanol solution (1×10^{-3} M) for 15 min followed by drying in the oven. Detection formaldehyde using the filter paper was carried out by two drops different concentrations of formaldehyde solution, i.e., 1.4, 1.6, 1.8, 2 M, and saturated solution (13.3 M) on the surface of the papers, respectively. Distilled water was also dropped to the paper strip as a negative control. Detection of formaldehyde in a vapor phase was performed by putting paper strip above the formaldehyde solution (1 M) in a vial and water was used as the negative control without dipping it in the solution. To confirm the detection ability of the paper strip, meatballs were tested by paper strip loaded with CHA. First, the meatballs were immersed in 1 M formaldehyde solution for overnight. The meatballs were then weighed (1 g), crushed with a mortar, and distilled water (10 mL) was added followed by filtration process. At last, the filtrate obtained was dropped onto the paper strip loaded with CHA compound.

RESULTS AND DISCUSSION

UV-VIS ABSORPTION STUDY OF CHA

The CHA chemosensor was synthesized by condensation of 6-nitroveratraldehyde with malononitrile to give CHA-I which upon reduction gave an symmetrical azine containing the amine group. The structure of prepared compounds have been fully characterized by FT-IR, 1H NMR, 13C NMR, and EI-MS analysis and agreed with the literature. The response of CHA as a sensor was examined by adding 10 μL of formaldehyde into a solution of CHA in ethanol. The color of the CHA solution changed from yellow to red immediately after addition of formaldehyde. This color change was confirmed using UV-Vis spectra as presented in Figure 2. The UV-Vis spectra showed that CHA ethanol solution has maximum wavelength of 412 nm which shifted to maximum wavelength of 509 nm after addition of formaldehyde. The color change of CHA solution after the addition of formaldehyde is the result of chemodosimeter type between chemosensor CHA and formaldehyde as Figure 3.

The -NH₂ of CHA probe as a binding group reacts with formaldehyde to form an imine. The formation of imine (-C = N-) induced extended delocalization of the electrons and the yellow color changed to red color (bathochromic shift). As shown in Figure 4, the spectra showed the peak of amine proton of CHA before addition formaldehyde solution that appeared at δ 6.8 ppm. After addition of formaldehyde, two new peaks were observed at δ 5.8 and 6.1 ppm, which indicate as the imine proton, the result of the reaction of the amine group in CHA with formaldehyde. Two methylene proton in imine structure have a different chemical environment, and the new peaks which appeared with high intensity in the range of δ 3-5 ppm were proton peaks of formaldehyde (Lewicki et al. 2015; Wu et al. 2018). Increase of formaldehyde addition from 80 to 200 equivalent reduced peak at δ 6.8 ppm and increased peak at δ 5.8 and 6.1 ppm.

The stability of CHA-Formaldehyde interaction was also studied by time dependence of UV-Vis spectra. The result indicated that after 3 h addition of formaldehyde the wavelength of 509 nm and 412 nm has decreased and the wavelength of 363 nm has appeared. The red color solution of CHA-formaldehyde has also changed to colorless (Figure 5).

As red color changed to colorless and wavelength shifted to hypsochromic, the imine group was hydrolyzed to aldehyde group. This result was also confirmed using NMR proton titration. As seen in Figure 4, the spectra of CHA-formaldehyde after 3 h addition appeared a new peak at 9.6 ppm. This peak indicated as a proton from aldehyde which is the result of hydrolysis of imine.

In order to determine the colorimetric response of CHA to formaldehyde, titration measurements with various

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**FIGURE 2.** The UV-Vis spectra of CHA (2.5 × 10^{-5} M in ethanol) before and after addition of formaldehyde (200 equiv.). Inset: Photographs of compound CHA (1 × 10^{-4} M in ethanol) before (left) and after addition of formaldehyde (right)

**FIGURE 3.** Reaction of CHA with formaldehyde in ethanol

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**FIGURE 4.** The spectra of CHA-formaldehyde after 3 h addition appeared a new peak at 9.6 ppm. This peak indicated as a proton from aldehyde which is the result of hydrolysis of imine.
and declining at the wavelength of 412 nm. Then, the limit of detection was calculated with the UV-Vis data, using formula as IUPAC recommendations, LOD: $3\sigma/m$, where $\sigma$ represents the standard deviation of blank, $m$ is slope of the equation that obtained from the ratio of absorption at 509 nm and 412 nm ($A_{509}/A_{412}$) versus concentration of formaldehyde ($HCHO$) (Borase et al. 2016), LOD of CHA was 0.74 M.

**FLUORESCENCE RESPONSE OF CHA TOWARD FORMALDEHYDE**

The fluorescent properties of CHA ($2.5 \times 10^{-4}$ M in ethanol) in the absence and presence of formaldehyde were determined. The CHA solution showed a strong fluorescence property, but after addition of formaldehyde, CHA was almost non-fluorescent. Chemosensor of CHA presented a strong absorption band at $\lambda=440$ nm and was chosen as the excitation wavelength for fluorescence measurement. At $\lambda_{ex}=440$ nm, the CHA solution demonstrated a maximum emission spectrum at 506 nm. Formaldehyde solutions at different concentrations (0-200 equiv.) were added concentrations of formaldehyde (0-200 equiv.) were carried out. As shown in Figure 6, increasing the formaldehyde concentration leads to the enhancing of absorption at 509 nm, and declining at the wavelength of 412 nm. Then, the limit of detection was calculated with the UV-Vis data, using formula as IUPAC recommendations, LOD: $3\sigma/m$, where $\sigma$ represents the standard deviation of blank, $m$ is slope of the equation that obtained from the ratio of absorption at 509 nm and 412 nm ($A_{509}/A_{412}$) versus concentration of formaldehyde ($HCHO$) (Borase et al. 2016), LOD of CHA was 0.74 M.

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into the CHA chemosensor in ethanol solution (2.5 × 10^-4 M) and the results were shown in Figure 7. As shown in Figure 7, when the addition of formaldehyde concentration increased, the fluorescence intensity of the compound CHA was decreasing. The result suggested that formation of imine quenched the fluorescence intensity, implying that CHA could be used as a ‘turn-off’ chemosensor for formaldehyde.

The limit of detection was calculated from the plot of $F_0/F$ versus concentration of formaldehyde (HCHO), where $F_0$ and $F$ is the fluorescence intensity of CHA in the absence of formaldehyde and at a certain concentration of formaldehyde, respectively. The Stern-Volmer plot in Figure 7 presented a linear relationship with $R^2=0.9658$ and the LOD was estimated to be 0.20 M.

To study for possible interference with other types of aldehydes in an application of CHA as the formaldehyde chemosensor, we measured the fluorescence intensity measurement of CHA after the addition of different aldehydes. As seen in Figure 8, except for glutaraldehyde, there was no interference effect of other aldehydes in the detection of formaldehyde.

**APPLICATION OF CHA AS PAPER STRIP**

A simple paper test was developed to demonstrate the practical application of CHA chemosensor for detecting formaldehyde visually. As shown in Figure 9, the paper dropped with a saturated water solution of formaldehyde (1 drop) changed its color from yellow to reddish. Whereas, the paper strip with water as negative control had no noticeable color change that could be observed by the naked eye. Application of CHA for detection formaldehyde as paper strip was also prepared by dropping formaldehyde at the various concentrations into paper loaded CHA and observed it under 365 nm portable UV lamp. The results (Figure 8) showed that the fluorescence was completely quenched with gradually increasing concentration of formaldehyde.

**SELECTIVITY STUDY**

![Figure 7](image1.png)  
**FIGURE 7.** Emission spectra of CHA (2.5 × 10^-4 M in ethanol) upon addition of formaldehyde in aqueous solution (0-200 equiv.) with excitation at 440 nm. Inset: fluorescence intensity as a function of formaldehyde concentration; photographs of compound CHA (2.5 × 10^-4 M in ethanol) before (left) and after (right) addition of formaldehyde under 365 nm portable UV lamp (A). Stern-Volmer plot of CHA against concentrations of HCHO (B)

![Figure 8](image2.png)  
**FIGURE 8.** Fluorescence intensity of CHA (1×10^-4 M in ethanol) at 506 nm after addition of different aldehyde compounds (200 equiv.) (blue bars); the mixture of CHA and formaldehyde after addition of other aldehyde compounds (red bars)
formaldehyde. Furthermore, the test paper which had been loaded with CHA and exposed to formaldehyde in gas phase turned to reddish, while those who were exposed to water as a control did not change.

Then, to demonstrate the applicability of paper strip for formaldehyde detection directly, meatball as a food sample was tested. As shown in Figure 9, paper strip added meatball filtrate containing formaldehyde changed from yellow to red quickly and the appearance was quenched. Based on these results, it can be concluded that the paper strip of CHA can be used to detect formaldehyde in the form of solutions and gases phases qualitatively.

CONCLUSION

In conclusion, a colorimetric and fluorometric chemosensor CHA for formaldehyde detection has been developed with high selectivity and sensitivity. Moreover, the sensor exhibits an obvious color change from yellow to red after addition of formaldehyde quickly. In this work, compound CHA was successfully applied as paper strip for formaldehyde detection.

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