Talanta 87 (2011) 276–283

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Dual fluorescence and electrochemical detection of the organophosphorus pesticides—Ethion, malathion and fenthion

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ARTICLE INFO

Article history:
Received 4 July 2011
Received in revised form 10 October 2011
Accepted 13 October 2011
Available online 19 October 2011

Keywords:
Organophosporus pesticides
Molecular sensors
Fluorescence
Electrochemistry

ABSTRACT

Organophosphorus (OP) based pesticides are known powerful inhibitors of cholinesterases, thus the toxicity of this class of compounds causes serious environmental and human health concerns. We report that benzodipyrido[3,2-a:2′,3′-c]phenazine (BDPPZ) and 3,6-dimethylbenzodipyrido-[3,2-a:2′,3′-c]phenazine (DM-BDPPZ) provide independent fluorescent and electrochemical signal transductions in the presence of the organophosphorus (OP) pesticides; fenthion, malathion and ethion. The presence of the methyl groups at the 3 and 6 positions in DM-BDPPZ was found to significantly influence the sensor performance. The difference in the fluorescence and electrochemical signals produced by the interaction of the sensor compound with each of the OP pesticides provides a means for differentiating between the three pesticides. Detection limits of 10^{-8} M, 10^{-9} and 10^{-12} M were obtained for fenthion, malathion and ethion, respectively. Due to the high sensitivity and ability to minimize false positives these new sensors will be useful for potential integration for future environmental use.

1. Introduction

Organophosphorus (OP) compounds with a thiophosphoryl (P=S) functional group constitute a broad class of widely used pesticides. According to the United States Environmental Protection Agency (EPA), about 70% of the insecticides in current use in the US are OP pesticides [1]. These OP pesticides are structurally related to the more reactive phosphoryl (P=O) organophosphates, which include lethal nerve agents and chemical warfare agents, such as, VX, Soman and Sarin. Unfortunately, frequent use of OP compounds in agricultural lands worldwide has resulted in their presence as residuals in crops, livestock, and poultry products and has further led to their migration into underground aquifers [2–4]. These compounds are highly toxic to human health and are powerful inhibitors of cholinesterase enzymes [5]. OP compounds poison thousands of humans across the world each year. In 1994, an estimated 74,000 children were involved in common household pesticide related exposures in the United States [1]. In a more recent study it was found that children exposed to OP pesticides were more likely to be diagnosed with attention deficit hyperactivity disorder (ADHD) [6].

Significant advances toward the development of detection methods for OP pesticides that include gas, liquid, and thin layer chromatography [7,8], immunoassays [9,10], nanoparticles [11–18] and biosensors based on inhibition of cholinesterase activity [19–25] have been reported. From a practical perspective, there is a continued need to obtain OP sensors that are portable, sensitive, selective and operate in real-time. Inhibition-based biosensors [3,26–28] have shown the most promise for detection of OP pesticides, however, there is a need to develop new materials that are robust under changes in changes in environmental conditions, for example temperature and pH [29,30]. Both soil and water are likely to contain OP pesticides due to heavy urban and rural use [30–33]. The broad range in toxicity levels of OP pesticides necessitates new methods to discriminate between various OP pesticides. Unfortunately, to date there have been no reports to demonstrate molecular sensors that differentiate selectively between OP pesticides. Here, we report that the highly conjugated phenanthroline derivatives benzodipyrido[3,2-a:2′,3′-c]phenazine (BDPPZ) and 3,6-dimethylbenzodipyrido-[3,2-a:2′,3′-c]phenazine (DM-BDPPZ) (Fig. 1) overcome the limitations of biosensors and further differentiate between the well-known OP pesticides *fenthion* (O,O-dimethyl O-4-methylthio-m-tolyl phosphorothioate), *ethion* (O,O,O′,O′-tetraethyl S,S′-methylene bis(phosphorodithioate)), and *malathion* (O,O-dimethyl S-[1,2-bis(ethoxycarbonyl) ethyl] dithiophosphate), Fig. 2.

2. Experimental

2.1. Materials and instrumentation

1,10-Phenanthroline, 2,9-dimethyl-1,10-phenanthroline, potassium bromide, sulfuric acid, nitric acid, fenthion, ethion and malathion, were obtained from Sigma–Aldrich.

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doi:10.1016/j.talanta.2011.10.015

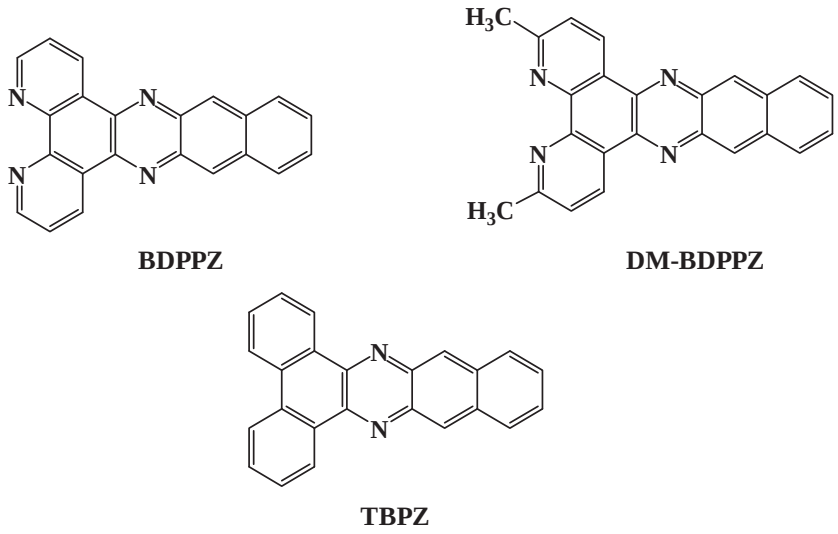

Fig. 1. Chemical structures of (a) benzodipyridophenazine (BDPPZ), (b) 3,6-dimethyl benzodipyridophenazine (DM-BDPPZ) and (c) tribenzophenazine (TBPZ).

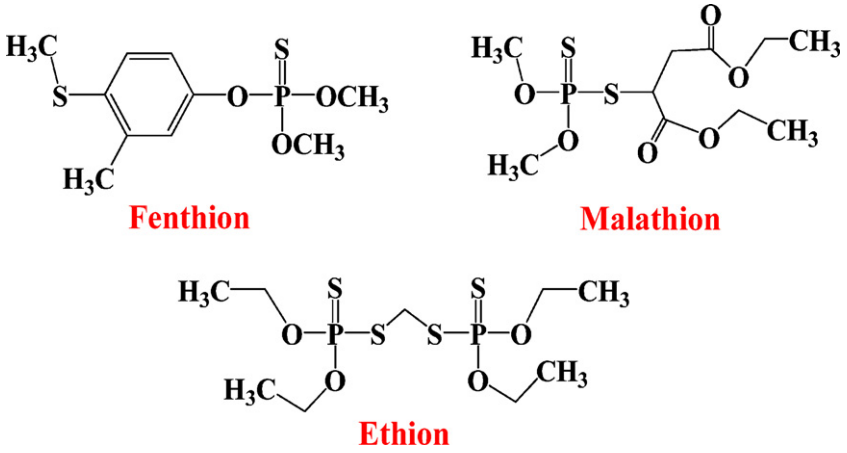

Fig. 2. Chemical structures of fenthion, ethion and malathion.

2,3-Diaminonaphthalene was obtained from Alfa Aesar. Tetrabutylammonium hexafluorophosphate ($TBAPF_6$) was obtained from Fluka. All solvents were obtained from Sigma–Aldrich and were of HPLC grade or better. All solvents were dried prior to use. UV–visible absorbance spectra were acquired using a Varian Cary 50 spectrophotometer. Emission spectra were acquired using a Varian Eclipse spectrofluorometer. ^{1}H NMR spectra were recorded on a 400 MHz JEOL spectrometer at room temperature. Differential pulse voltammertric (DPV) measurements were carried out using a three-electrode arrangement on a CV 50 BAS electrochemical analyzer. A glassy carbon electrode was chosen as the working electrode, the counter-electrode was a platinum wire and the reference electrode was Ag/AgCl (3 M KCl). The supporting electrolyte solution was 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$) in acetonitrile. All electrochemical measurements were carried out in a N_2 saturated atmosphere.

2.2. Synthetic procedures

BDPPZ and DM-BDPPZ were synthesized following a modified literature procedure [34–36]. 1,10-Phenanthroline and 2,9-dimethyl-1,10-phenanthroline were oxidized to their corresponding dione, followed by coupling with 2,3-diaminonaphthalene in a condensation reaction. Details are provided below.

2.2.1. Synthesis of 1,10-phenanthroline-5,6-dione

20 mL of concentrated sulfuric acid (H_2SO_4) and 10 mL of nitric acid (HNO_3) were added dropwise to a mixture of 1,10-phenanthroline (1.00 g, 5.56 mmol) and KBr (5.95 g, 50 mmol) at 0 °C. The mixture was refluxed at 80 °C for 2 hrs, then cooled to room temperature. The contents of the reaction flask were diluted with deionized water (400 mL), and neutralized with sodium bicarbonate ($NaHCO_3$). The product was extracted with methylene chloride, and dried over anhydrous $MgSO_4$. After all solvents were removed on a rotary evaporator, the product was concentrated in vacuum, resulting in a yellow solid. Purification took place by recrystallization from methanol. The average yield was 95% (1.11 g, 5.31 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.12–9.10 (*t*, 2H, *J* = 2.85 Hz), 8.51–8.48 (*d*, 2H, *J* = 1.83 Hz), 7.60–7.55 (*m*, 2H, *J* = 4.71 Hz).

2.2.2. Synthesis of benzo[i]dipyrido-[3,2-a:2′,3′-c]phenazine (BDPPZ)

1,10-Phenanthroline-5,6-dione (0.50 g, 2.38 mmol) was refluxed in ethanol for 15 min. 2,3-Diaminonaphthalene (0.38 g, 2.38 mmol) was added to the dione and the mixture was allowed to reflux for 4 h. The solution color changed from yellow to orange. The solution was allowed to cool to room temperature and then filtered to collect the solid product. The product was washed with methanol, and concentrated in vacuum. The average reaction

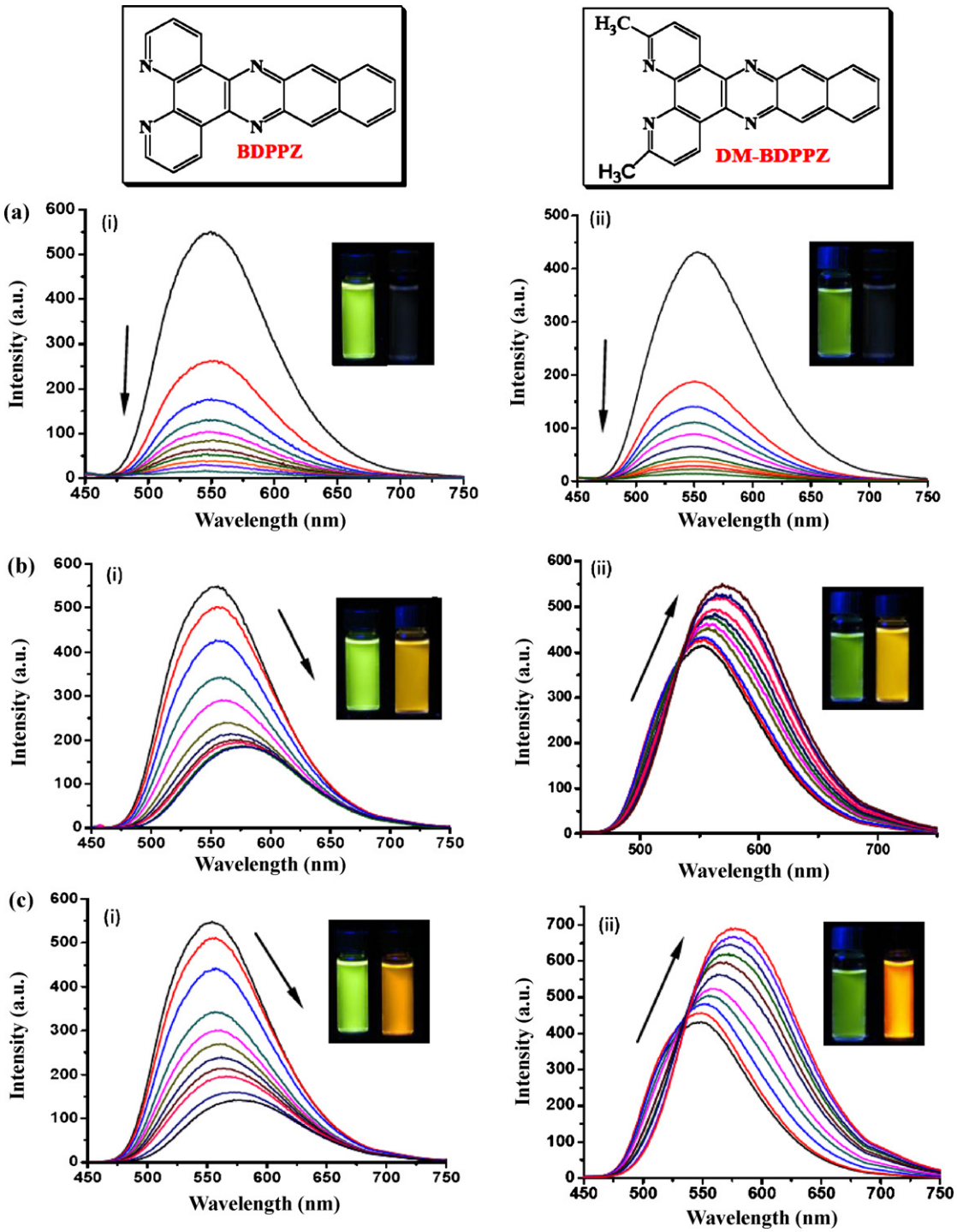

Fig. 3. Changes in the fluorescence spectra of (i) BDPPZ (ii) DM-BDPPZ when titrated with (a) fenthion, (b) malathion, and (c) ethion, respectively. The insets show the corresponding color changes. In each case the direction of the arrow indicates concentration of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM.

yield was 80%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.73–9.70 (*d*, 2H, J = 7.30 Hz), 9.33–9.32 (*d*, 2H, J = 2.96 Hz), 8.99 (*s*, 2H), 8.24–8.22 (*d*,*d*, 2H, J = 2.92 Hz), 7.87–7.84 (*q*, 2H, J = 4.40 Hz), 7.67–7.64 (*d*,*d*, 2H, J = 3.28).

2.2.3. Synthesis of 2,9-dimethyl-1,10-phenanthroline-5,6-dione

Concentrated H_2SO_4 (20 mL) and concentrated HNO_3 (10 mL) were added to 2,9-dimethyl-1,10-phenanthroline (1.16 g, 5.56 mmol) at 0 °C. The mixture was allowed to reflux at 80 °C for 2 h then cooled to room temperature. The contents of the reaction flask were diluted with deionized water (400 mL), and neutralized with sodium bicarbonate ($NaHCO_3$). The product was extracted with methylene chloride, dried over anhydrous $MgSO_4$, and was concentrated in vacuum, resulting in a yellow-brown solid. Purification was accomplished by recrystallization from methanol to yield yellow-brown crystals. The average yield for this reaction was 95%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.38–8.35 (d, 2H, J = 7.98 Hz), 7.42–7.39 (d, 2H, J = 8.01 Hz), 2.84 (s, 6H).

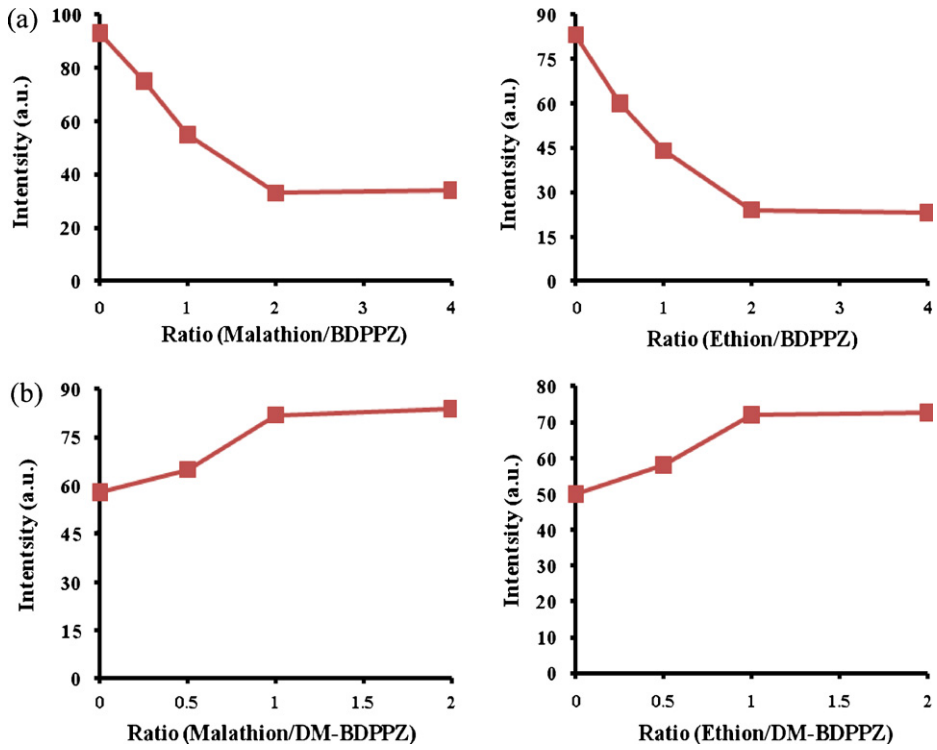

Fig. 4. Continuous variation of the ratios of (a) BDPPZ and (b) DM-BDPPZ with malathion and ethion. BDPPZ shows a 1:2 sensor:pesticide ratio, while DM-BDPPZ shows a 1:1 sensor:pesticide ratio.

2.2.4. Synthesis of 3,6-dimethyl-benzo[i]dipyrido-[3,2-a:2′,3′-c]phenazine (DM-BDPPZ)

2,9-Dimethyl-1,10-phenanthroline-5,6-dione (1.13 g, 4.78 mmol) was refluxed in ethanol for 15 min. After all the dione had dissolved, 1 equiv. of 2,3-diaminonaphthalene (0.755 g, 4.78 mmol) was added to the contents of the reaction flask. The reaction was refluxed for 4 h. During the course of the reaction the solution color changed from yellow to orange. The solution was allowed to cool to room temperature and then filtered to collect the solid product. The product was washed with methanol and concentrated in vacuum. Purification was accomplished by recrystallization from methanol to yield red crystals. DM-BDPPZ was produced in approximately 75–80% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.56–9.54 (d, 2H, J = 8.40 Hz), 8.94 (s, 2H), 8.21–8.20 (m, 2H, J = 3.32 Hz), 7.67–7.64 (d, 2H, J = 8.44 Hz), 7.62–7.60 (m, 2H, J = 3.28 Hz), 2.9–3.0 (s, 6H).

2.2.5. Synthesis of tribenzo[a,c,i] phenazine

9,10-Phenanthrenequinone (0.937 g, 4.50 mmol) was dissolved in 20 mL of ethanol, and refluxed for 10 min at 80 °C. One equivalent of 2,3-diaminonaphthalene (0.712 g, 4.50 mmol) was dissolved in 20 mL of ethanol, and refluxed for 10 min at 80 °C. Two solutions were mixed and refluxed for 2 h. The solution was allowed to cool to room temperature and then filtered to collect the solid product. The product was washed with methanol, and concentrated in vacuum. Purification was accomplished by recrystallization from methanol to yield a yellow-orange solid. Tribenzo[a,c,i]phenazine was produced in approximately 90% yield. ^{1}H NMR (THF-d_8, 400 MHz) δ: 9.52–9.54 (d, 2H, J = 6.6 Hz), 9.05 (s, 2H), 8.75–8.76 (d, 2H, J = 8.1 Hz), 8.33, 7.69 (AA′BB′, 4H), 7.82 (m, 4H).

2.2.6. Electrochemical measurements

Voltammetric measurements were carried out through a three-electrode arrangement using a BAS Epsilon electrochemical analyzer. Differential pulse voltammetry (DPV) data of BDPPZ and DM-BDPPZ were recorded in acetonitrile solution containing 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$) as supporting electrolyte in the absence and presence of three OP pesticides. Measurements were taken under a N_2 atmosphere. A glassy carbon electrode was chosen as the working electrode, while the counter-electrode was a platinum wire and the reference electrode was Ag/AgCl (3 M KCl). Differential pulse voltammograms of 1.8×10^{-4} M BDPPZ or DM-BDPPZ solutions alone and with the addition of 2 equiv. of each pesticide, were obtained at a 0 to −1500 mV (*vs.* Ag/AgCl) potential window at a scan rate of 20 mV s^{-1} and pulse amplitude of 50 mV.

3. Results and discussion

3.1. UV–visible absorbance and emission spectra

The absorption spectrum of BDPPZ shows two absorption peaks at 390 nm and 410 nm in acetonitrile solution. DM-BDPPZ also shows two absorption peaks centered at 395 nm and 415 nm. For both BDPPZ and DM-BDPPZ, an excitation wavelength of 385 nm was used for emission measurements. The emission spectra of both BDPPZ and DM-BDPPZ obtained in acetonitrile solvent showed a peak centered at 550 nm. The emission quantum yields of BDPPZ and DM-BDPPZ were measured in acetonitrile and found to be 0.15 and 0.12, respectively, using coumarin 153 as a standard. The interaction of the BDPPZ and DM-BDPPZ with three OP pesticides, fenthion, malathion and ethion, was investigated.

3.2. Titration with OP pesticides

A 1.4×10^{-5} M solution of BDPPZ or DM-BDPPZ was prepared in acetonitrile and titrated with each of the OP pesticides, while monitoring changes in emission spectra. In each case, an acetonitrile solution of each of the sensor molecules was freshly prepared at

room temperature. Emission measurements were taken following addition of each aliquot of OP pesticide added. All measurements were taken at room temperature.

Fig. 3a(i and ii) shows the changes in emission spectra of BDPPZ and DM-BDPPZ titrated with fenthion. In both cases the emission of the sensor molecules was completely quenched. The detection limit in both cases was 1×10^{-8} M fenthion. On the other hand, titration of BDPPZ with malathion resulted in emission quenching, but this time the quenching was accompanied by a 20 nm red-shift from 550 nm to 570 nm (Fig. 3b(i)). At the end of the titration, the fluorescence color had changed from green to yellow and the solution was dimmer as shown in the corresponding photograph in Fig. 3b(i). Surprisingly, the titration of DM-BDPPZ with malathion did not result in fluorescence quenching as observed in the case of BDPPZ. Instead, the fluorescence intensity increased and was accompanied by a 25 nm red-shift from 550 nm to 575 nm (Fig. 3b(ii)). The fluorescence color of the solution changed from green to yellow and was brighter as shown in the corresponding photograph. The detection limit for BDPPZ and DM-BDPPZ with malathion is 1×10^{-8} M and 1×10^{-9} M, respectively. Similar behavior was observed when BDPPZ and DM-BDPPZ were titrated with ethion. Titration of BDPPZ with ethion resulted in emission quenching accompanied by a 30 nm red-shift from 550 nm to 580 nm (Fig. 3c(i)), while in the case of DM-BDPPZ titrated with ethion, the emission intensity increased and was accompanied by a 35 nm red-shift from 550 nm to 585 nm (Fig. 3c(ii)). The detection limit for BDPPZ and DM-BDPPZ with ethion is 1×10^{-12} M and 1×10^{-11} M, respectively. We emphasize that there were no further shifts in wavelength or changes in intensity of both sensor molecules at saturation concentrations of each of the corresponding OP pesticides. We further note that there were no changes in the absorbance spectra of BDPPZ or DM-BDPPZ when titrated with either of the OP pesticides at saturation concentrations.

3.2.1. Calculation of binding constants

Despite the structural similarities between BDPPZ and DM-BDPPZ, the DM-BDPPZ methyl groups significantly influence the interaction of the 'phenanthroline nitrogens' with the OP compounds. The method of continuous variation showed that DM-BDPPZ forms a 1:1 complex with fenthion, malathion and ethion. On the other hand, BDPPZ was found to form a 1:2 complex with ethion and malathion, but a 1:1 complex with fenthion, Fig. 4

Binding constants were calculated based on a 1:1 or 1:2 sensor:analyte complex [37]. For both BDPPZ and DM-BDPPZ, fenthion acts as the quencher. The emission quenching data for BDPPZ and DM-BDPPZ with fenthion were analyzed using the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where Q is the quencher, F_0 and F are the emission intensity in the absence of a quencher and in the presence of the quencher at [Q] concentration, respectively, and K_{SV} is the Stern–Volmer dynamic quenching constant.

For this study, the quenching occurs within a sensor–analyte complex, in which case the Stern–Volmer constant, K_{SV} is equal to the association constant, K_A. The Stern–Volmer plot for quenching of both BDPPZ and DM-BDPPZ by fenthion showed good linearity in the range of quencher concentration used in our experiments, and provided the binding constant for the association of sensor molecules and fenthion. Based on the calculations, the binding constants of fenthion with BDPPZ and DM-BDPPZ were found to be $413.2 \pm 2.0\ M^{-1}$, and $808.5 \pm 10.3\ M^{-1}$, respectively.

The binding constant (K) was calculated from the emission titration data of both BDPPZ and DM-BDPPZ with malathion and ethion. The basis for the calculation of the binding constant is a 1:1 or 1:2

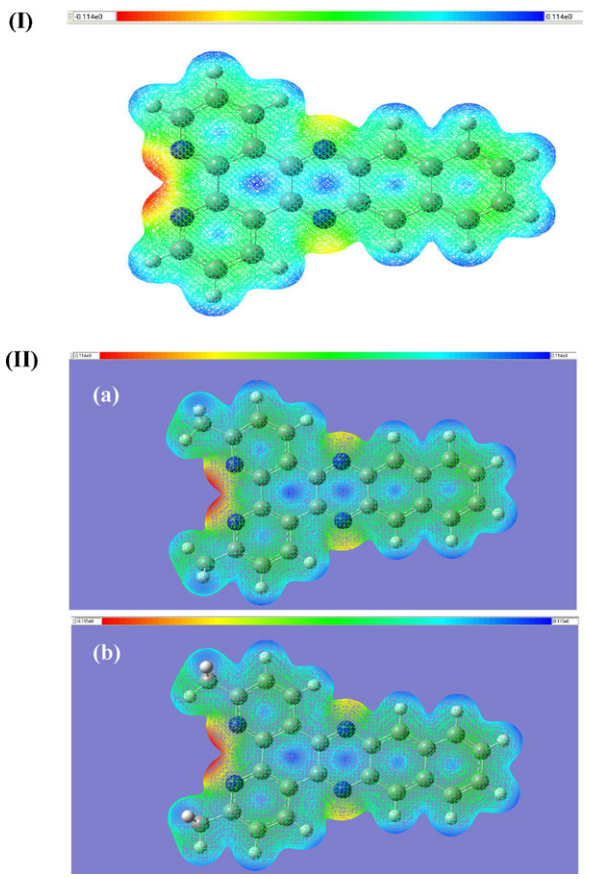

Fig. 5. (I) Electron density of BDPPZ from total SCF with the isovalue 0.01. (II) Electron density of DM-BDPPZ from total SCF with the isovalue 0.01, (a) eclipsed structure, and (b) staggered structure.

interaction. As mentioned earlier, based on the method of continuous variation we found that BDPPZ forms a 1:2 complex with the OP pesticides; while for DM-BDPPZ forms a 1:1 complex with the OP pesticides. The 1:2 interaction of sensor and analyte dictates that K takes the form as listed below in Eq. (1) where L abbreviates sensor molecules and G abbreviates OP pesticides. The emission data was then used to calculate K_A. The resulting values were averaged and reported with the respective standard deviation.

$$L + 2G \rightleftharpoons LG + G$$

$$LG + G \rightleftharpoons LG_2$$

$$K_1 = \frac{[LG][G]}{[L][G]^2} \quad K_2 = \frac{[LG_2]}{[LG][G]}$$

$$K = K_1 \times K_2 = \frac{[LG][G]}{[L][G]^2} \times \frac{[LG_2]}{[LG][G]}$$

$$K = \frac{[LG_2]}{[L][G]^2}$$

$$K = \frac{[LG_2]}{([L] - [LG_2])([G] - [LG_2]^2/2)^2} \quad (1)$$

Based on the above equations, the binding constants for BDPPZ with malathion and ethion were found to be $3.1 \pm 0.3 \times 10^4\ M^{-2}$, $8.5 \pm 0.6 \times 10^4\ M^{-2}$, respectively.

The 1:1 interaction of sensor and analyte dictates that K takes the form as listed below in Equation 2 where L abbreviates sensor

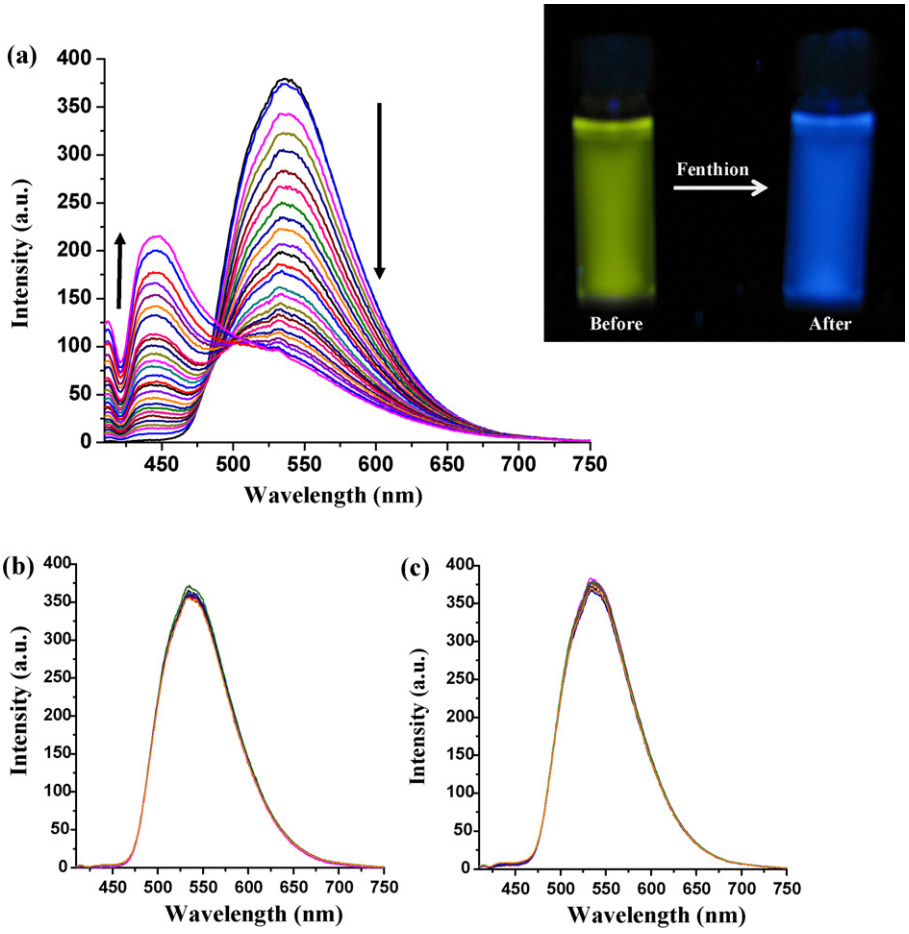

Fig. 6. Changes in emission spectrum of tibenzo[a,c,i]phenazine (TBPZ) titrated with (a) fenthion, the direction of the arrow indicates concentrations of 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5 μM. (b) 20 μM malathion, and (c) 20 μM ethion. The photograph in the inset of (a) shows the color changes associated with the change in emission spectrum of TBPZ.

molecules and G abbreviates OP pesticides. The emission data was then used to calculate K. The resulting values were averaged and reported with the respective standard deviation.

$$L + G \Leftrightarrow LG$$

$$K = \frac{[LG]}{[L][G]} = \frac{[LG]}{([L] - [LG])([G] - [LG])} \quad (2)$$

Based on the above equations, the binding constants for DM-BDPPZ with malathion and ethion were found to be $4.2 \pm 0.1 \times 10^3\ M^{-1}$, $1.5 \pm 0.4 \times 10^4\ M^{-1}$, respectively.

3.3. Computational calculations

To gain better insight of the interactions of BDPPZ and DM-BDPPZ with the OP pesticides as well as the binding mechanism, computational calculations were performed (Fig. 5). The calculations were carried out using the Gaussian 03 program suite [38]. The structures of both BDPPZ and DM-BDPPZ were optimized at the B3LYP level of density functional theory. Initially the structures were optimized using restricted Hartree–Fock method and a small basis set. These structures were then used as the starting point for the density functional calculations. Further optimizations were performed using the 6−31G basis set, expanded to include polarization and diffuse functions (6−31 + G(d,p)). Frequency calculations at the same level of theory were also performed to confirm that all stationary points were minima (no imaginary frequencies).

The calculation results showed that the phenanthroline nitrogens of BDPPZ and DM-BDPPZ have higher electron density relative to the phenazine nitrogens. Therefore, we hypothesized that binding of BDPPZ and DM-BDPPZ to the OP pesticides occurred between the phenanthroline nitrogens and the phosphorus atoms, and in doing so, displace the leaving group of the OP pesticide. It is well-known that the mechanism by which choline esterase-based enzymes bind to OP compounds is through the phosphorus atom followed by displacement of the leaving group [39]. We hypothesize that the leaving group becomes the counter anion of the complex formed.

3.4. Interaction of TBPZ with fenthion, malathion and ethion

In an effort to further investigate the binding mechanism between the sensor molecules and the OP pesticides, we synthesized the molecule tribenzo[a,c,i]phenazine (TBPZ), (structure (c) shown in Fig. 1) and examined changes in its fluorescence spectra upon interaction with ethion, malathion and fenthion. The rationale for using TBPZ as a control experiment is that its structure lacks the phenathroline nitrogens that are present in both BDPPZ and DM-BDPPZ, and thus allows us to examine the effects of only the phenazine nitrogens.

A 1.4×10^{-5} M solution of TBPZ was prepared in acetonitrile and titrated with each of the OP pesticides, while monitoring changes in fluorescence spectra. Fig. 6 shows the results of the titration of TBPZ with fenthion. It was observed that increase in fenthion

concentration resulted in quenching of the TBPZ emission peak centered at 535 nm, and was accompanied by the emergence of a new peak at 450 nm. At the end of the titration, the fluorescence color of TBPZ had changed from green to blue as shown in the corresponding photograph in Fig. 6a. Fig. 6b and c shows the results obtained when TBPZ was titrated with malathion and ethion, respectively. In each case, there were no notable changes in the emission spectra of TBPZ. We note that no changes in the UV–visible absorbance spectra of TBPZ were observed when titrated with fenthion, malathion or ethion.

The results showed that addition of saturation concentrations of ethion and malathion to TBPZ do not influence the emission spectra, however, addition of fenthion strongly affects the emission spectra by causing shifts in emission intensity and wavelength. The results, thus suggest that ethion and malathion bind to BDPPZ and DM-BDPPZ through the phenanthroline nitrogens, while fenthion interacts with BDPPZ and DM-BDPPZ through the phenazine nitrogens.

We further examined the changes in fluorescence intensity of the BDPPZ and DM-BDPPZ when they were titrated with the electrophile H^+ (in this case, HCl was chosen). As shown in Fig. 7 , the addition of HCl produces a significant response, which is identical for BDPPZ and DM-BDPPZ, The emission spectra of both BDPPZ and DM-BDPPZ was quenched and accompanied by a red-shift in the presence of H^+. Furthermore, it was found that HCl formed a 1:2 BDPPZ:H^+ complex and DM-BDPPZ:H^+ complex. The fact that the fluorescence of both BDPPZ and DM-BDPPZ was quenched due to a 1:2 complexation is additional evidence that the reason we observe fluorescence enhancement of BDPPZ when bound to ethion and malathion is due to a 1:1 complexation. Thus, the difference in behavior of both BDPPZ and DM-BDPPZ with each of the OP pesticides investigated is reflective of the structure of the complex formed upon binding. The results indicate that the difference in emission behavior results from differences in the complexes' structural forms.

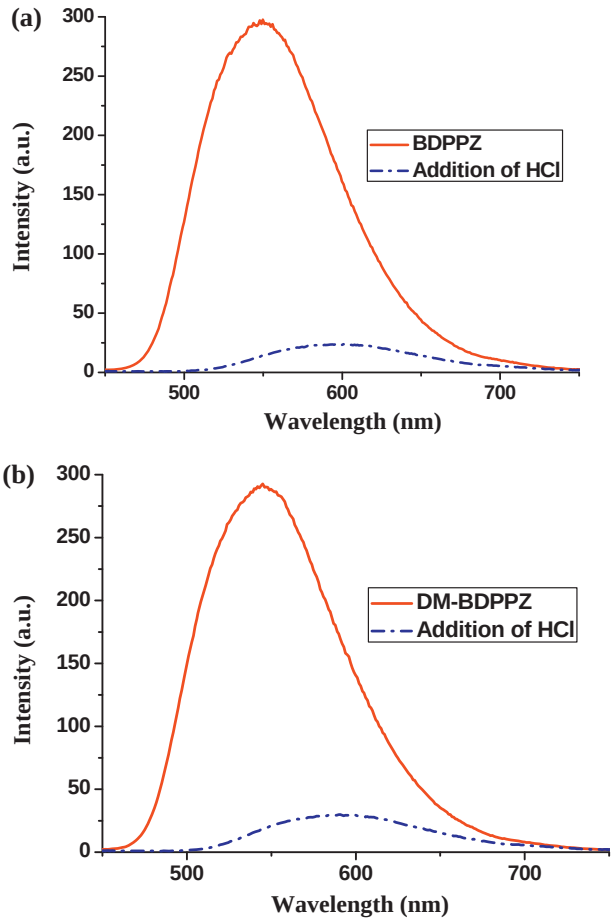

Fig. 7. Changes in fluorescence spectra of (a) BDPPZ before and after addition of 2 equiv. by molar concentration of HCl and (b) DM-BDPPZ before and after addition of 2 equiv. by molar concentration of H^+. In each case the fluorescence is quenched and red-shifted.

3.5. Electrochemical detection

While the fluorescence measurements showed that DM-BDPPZ was selective and sensitive toward three OP pesticides, it was important to determine whether an alternative signal output could be provided as a result of binding. From a general viewpoint, sensors that provide dual independent signal outputs are advantageous as they minimize the risk of false-positive signals. Furthermore, we aimed to investigate whether electrochemical measurements of BDPPZ and DM-BDPPZ when bound to OP pesticides would provide an alternative signal output for sensing of OP pesticides. Differential pulse voltammertry (DPV) was used to investigate interactions of the OP pesticides with BDPPZ and DM-BDPPZ. As shown in Fig. 8 and Table 1, significant changes were observed upon addition of excess pesticides. We note that the DPV data shown excludes any influence of the OP pesticides alone.

The differential pulse voltammogram of a 1.8×10^{-4} M BDPPZ solution showed two reduction peaks at −0.91 V and −1.36 V *vs.* Ag/AgCl as shown in Fig. 8a. Addition of up to 2 equiv. of fenthion did not affect the differential pulse voltammogram of BDPPZ. However, addition of 2 equiv. of malathion resulted in a peak shifts to positive potentials −0.48 V and −1.07 V *vs.* Ag/AgCl, respectively, while addition of 2 equiv. of ethion, resulted in two peaks at −0.09 V and −0.84 V *vs.* Ag/AgCl, respectively.

The differential pulse voltammogram of 1.8×10^{-4} M DM-BDPPZ in acetonitrile solution displayed peaks at −0.96 V and

Table 1
Summary of the (a) BDPPZ and (b) DM-BDPPZ sensors with various OP pesticides.

BDPPZ	[a]Before (nm)	[a]After (nm)	[b]DPV Before *vs.* Ag/AgCl	[b]DPV After *vs.* Ag/AgCl	Detection limit (M)
(a)					
Fenthion		550	−0.91, −.136	−0.91, −1.36	10^{-8}
Malathion	550	570		−0.48, −1.07	10^{-9}
Ethion		580		−0.09, −0.84	10^{-12}
DM-BDPPZ	[a]Before (nm)	[a]After (nm)	[b]DPV before *vs.* Ag/AgCl	[b]DPV after *vs.* Ag/AgCl	Detection limit (M)
(b)					
Fenthion		550	−0.96, −.130	−0.96, −1.30	10^{-8}
Malathion	550	575		−0.65, −1.18	10^{-8}
Ethion		585		−0.15, −0.82	10^{-11}

[a] The fluorescence peaks changes before and after the addition of OP pesticides.
[b] Differential pulse voltammertry data before and after the addition of OP pesticides. All measurements were made in acetonitrile solvent and at room temperature.

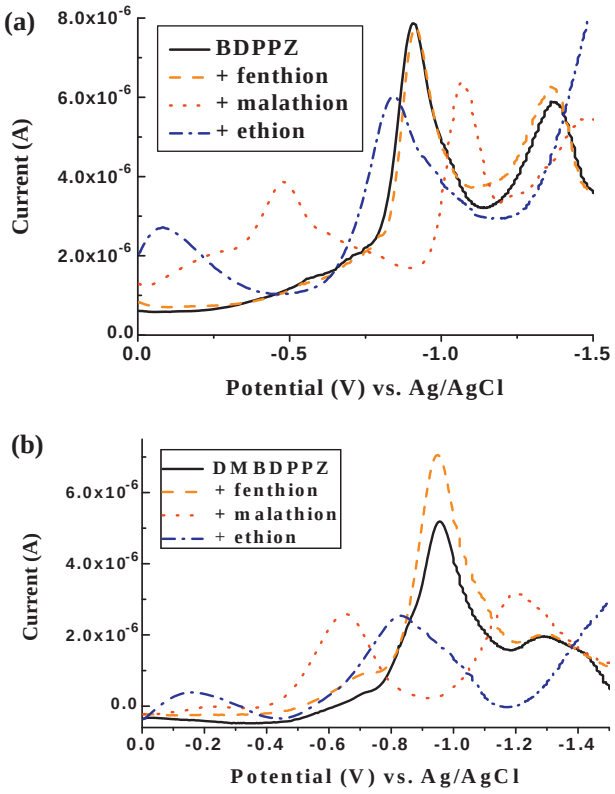

Fig. 8. (a) Changes in differential pulse voltammetry (DPV) of 1.8×10^{-4} M BDPPZ with 2 equiv. of fenthion, malathion or ethion. (b) Changes in differential pulse voltammetry (DPV) of 1.8×10^{-4} M DM-BDPPZ with 2 equiv. of fenthion, malathion or ethion.

−1.30 V *vs.* Ag/AgCl. Addition of 2 equiv. of fenthion caused increase in current, as shown in Fig. 8b. In the case of addition of 2 equiv. malathion, the DPV showed shifts to −0.65 V and −1.18 V *vs.* Ag/AgCl, while addition of 2 equiv. of ethion, caused shifts to −0.15 V and −0.82 V *vs.* Ag/AgCl. The emergence of new peaks in the DPV of BDPPZ and DM-BDPPZ indicates interaction with the OP pesticides. The difference in the peak positions further affirms the selectivity of the sensor, as well as the differences in binding mode.

4. Conclusions

The fluorescence and electrochemical changes of BDPPZ and DM-BDPPZ when exposed to fenthion, ethion or malathion shows the potential of phenanthroline derivatives as viable sensors with dual signal transductions. The sensor molecules show three significant features: (1) high fluorescence quantum yields, (2) remarkable spectral shifts or intensity changes in fluorescence when bound to OP pesticides, and that (3) the complexation with pesticides lead to shifts in redox behavior. These three features are essential toward the development of selective sensors for OP pesticides that yield different signal outputs characteristic of the nature of the organophosphorus compounds.

We have successfully designed a dual fluorescent and electrochemical sensor for OP pesticides. The sensors demonstrate the ability to distinguishing three various pesticides by providing different signals with each pesticide.

Acknowledgements

S.O.O. is grateful to the NSF for the CAREER award under grant CHE 1005456. W.G. is grateful to the Western Michigan University Graduate College for a Dissertation Completion Fellowship. T.L.J. acknowledges the American Chemical Society Project SEED program for funding. The authors wish to acknowledge partial financial support under U.S. Army Grant No. W911QY-07-1-0003.

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