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Spectrophotometric detection of organophosphate diazinon by porphyrin solution and porphyrin-dyed cotton fabric

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Abstract

The absorbance spectrum of porphyrin meso-tetraphenylporphine (TPP) shifts to a shorter wavelength when interacting with the organophosphate diazinon. This spectral shift in the presence of diazinon is more obvious in the difference spectra (TPP + diazinon) − TPP, and can be observed in porphyrin−DMF solution and porphyrin-dyed cotton fabric. In solution, the difference spectrum has a peak at 412 nm and a trough at 421 nm. For TPP dyed cotton fabric, the difference spectrum has a peak at 415 nm and a trough at 430 nm. The absorbance difference (ΔA) between peak and trough in the difference spectra has a linear relationship with diazinon concentration. This spectral property of porphyrin can be used to detect diazinon in the environment. Diazinon can be detected at 0.5 ppm level by TPP in solution, and at 11 ppm level by TPP dyed cotton fabric. The solid state detection capability of TPP dyed cotton fabric implies that textiles can serve as the platform for chemical sensors.

Keywords: Porphyrin; Organophosphate; Diazinon; Detection; Dyeing; Absorbance spectroscopy

1. Introduction

Organophosphate (OP) compounds are commonly used as pesticides and herbicides in agricultural industry and as chemical warfare agents in military practice. OPs can inhibit enzyme acetylcholinesterase (AChE) and cause permanent nervous system damage. One agricultural organophosphate pesticide, diazinon, was ranked 5th by the California Pesticide Illness Surveillance Program (1982−1995) as a cause of systemic poisoning in California from 1990 to 1994 [1]. In addition to industrial and agricultural incidents, OP chemical warfare agents such as nerve gas are capable of causing casualties in the range of hundreds to a few thousand. In 1995, a terrorist group launched an attack using sarin nerve gas against commuters in the Tokyo subway system. This highly publicized attack killed 12 people and affected over 5000 [2].

Detecting toxic OP chemicals is an area of extensive research for protecting farmers, first-responders, and military personnel. Traditional analytical techniques for OP detection such as gas, liquid, and thin-layer chromatography require expensive laboratory equipment, highly trained technicians and long detection times, yet, these techniques are very sensitive and reliable [3].

Many current OP detection research studies are focused on biosensor development, which is based on acetylcholinesterase (AChE) inhibition test or direct organophosphorus hydrolase (OPH) detection [4]. The enzyme AChE terminates impulse transmission at cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine to acetate and choline. The toxicity of OPs is due to the inhibition of AChE, resulting in the buildup of acetylcholine which interferes with muscular responses and in vital organs produces serious symptoms and eventually death [3,5]. Comparing catalytic rate of AChE or other esterases at a given time to a baseline, background or pre-exposure level provides a way to detect the existence of OPs that inhibit AChE. The inhibition of AChE activity can be measured by the change in pH associated

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with acetylcholine hydrolysis or by oxidizing the choline via choline oxidase and measuring the rate of oxygen consumption or hydrogen peroxide production. Other AChE inhibition sensors include the measurement of oxidation current of the electroactive thiocholine iodide using amperometric techniques [4]. Since AChE is inhibited by OPs as well as by carbamate pesticides and other compounds, sensors based on AChE inhibition are not selective [3].

OP pesticides such as parathion, coumaphos, and acephate can be catalytically hydrolyzed by OPH and specific products are generated from the hydrolysis. Two protons and a chromophoric and/or electroactive alcohol are generated from the OPH-catalyzed hydrolysis of organophosphates as a result of a cleavage of P–O, P–F, P–S or P–CN bonds [3]. By monitoring the chromophoric hydrolysis products, *p*-nitrophenol from paraoxon, parathion, methyl parathion, or chlorferon from coumaphos, the existence of OPs can be detected [3]. Mulchandani's group developed a series of OPH-based biosensors using potentiometric transducer such as a pH electrode [6], optical transducer to monitor the chromophoric products such as *p*-nitrophenol [7], or amperometric transducer to monitor the oxidation or reduction current of the hydrolysis products [8].

Porphyrins are reversible enzyme inhibitors for AChE [5] and OPH [9]. Displacing monosulfonate tetraphenyl porphyrin ($TPPS_1$) by another competitive inhibitor, tetracaine [10] or OP compound diazinon [11], causes spectrophotometric changes of $TPPS_1$. Addition of OP substrates of OPH, such as paraoxon, coumaphos, diazinon or malathion, displaces copper complexed meso-tri(4-sulfonato phenyl) mono(4-carboxy phenyl) porphyrin (CuC_1TPP) in OPH and results in spectral absorbance change of CuC_1TPP [9]. The spectrophotometric changes of porphyrin–enzyme complex in the presence of OPs can be used in OP detection protocol. In these studies [9,11], porphyrins ($TPPS_1$ and CuC_1TPP) and enzymes (AChE and OPH) were immobilized on glass slides to provide solid state detection of OPs.

For enzyme-based sensors, there are strict pH and temperature requirements for the storage to maintain the activity of the enzymes. Though OPH-based biosensors show long term stability, they have to be stored in buffer at 4 °C [7]. Without the presence of enzymes, porphyrins also have spectral shifts when bound with other chemicals [12,13]. This property of porphyrin was used in detection protocols for cyanide [14], amino acids [15], dipicolinic acid [16] and pentachlorophenol [17]. The first objective of this study is to investigate OP detection by porphyrin in the absence of enzymes.

The above mentioned chemical detection protocols by porphyrins [14–17] could be applied to cellulosic substrates to provide a solid state detection. The cellulosic substrates include cellulose film SPECTRA/POR® molecular-porous membrane [14,15], and cellulose fiber Kimwipes® paper tissue [16,17]. Although the textile dyeability of porphyrins has not been investigated, porphyrins have the potential to be used as textile dyes since they have a similar chemical structure as a very popular and important category of textile dyes, phthalocyanines, which deliver bright blue or green color. Porphyrins also have solid state detection potential when immobilized on cotton fabric because cotton has similar cellulosic structure as SPECTRA/POR® membrane and Kimwipes® paper. The second objective of this study is to dye/immobilize porphyrin on cotton fabric and investigate the solid state OP detection by porphyrin-dyed fabric.

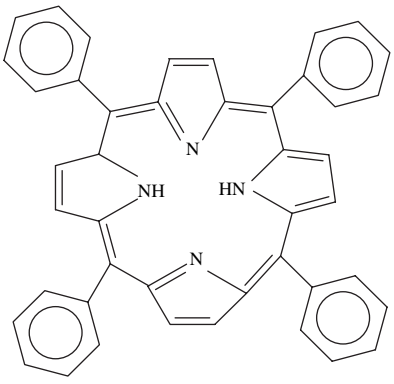
2. Experimental

2.1. Chemicals and materials

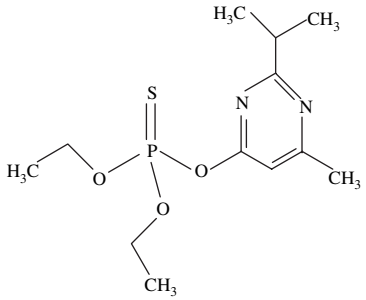
Porphyrin meso-tetraphenylporphine (TPP) was purchased from Frontier Scientific (Logan, UT) and used without further purification. OP compound diazinon was purchased from Sigma–Aldrich and used as received. Chemical structures of TPP and diazinon are shown in Fig. 1. Medium weight, plain weave 100% cotton fabric, which was singed, desized and bleached, was obtained from Cotton Incorporated (Cary, NC).

2.2. Methods

In liquid phase detection study, both TPP and diazinon were dissolved in organic solvent *N*,*N*-dimethylformamide (DMF). Absorption spectra of TPP in the presence and absence of diazinon were collected at room temperature using a Cary 300 UV–visible spectrophotometer (Varian Inc., Palo Alto, CA). Difference spectra were obtained by the subtraction of absolute spectra using Grams/AI (Thermo Galactic, Salem, NH). Peak positions in difference spectra were determined by



TPP



Diazinon

Fig. 1. Chemical structures of meso-tetraphenylporphine (TPP) and diazinon.

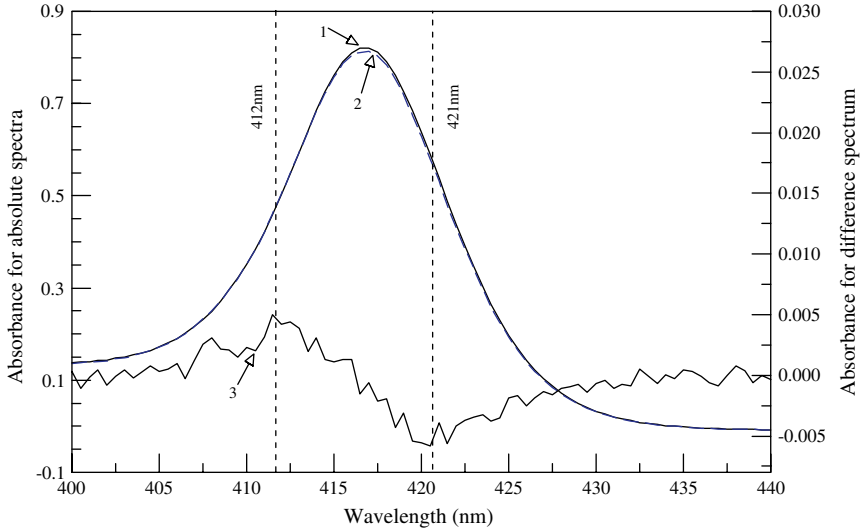


Fig. 2. The absorbance spectrum of TPP in the absence and presence of diazinon. The subtraction of the absolute spectrum of TPP (trace 1) from the absolute spectrum of TPP + diazinon (trace 2, dashed line) gives the difference spectrum (TPP + diazinon) − TPP (trace 3).

second derivative of the spectra using Grams/AI. Linear regression was performed using PSI-Plot (Poly Software International, Pearl River, NY).

Solvent dyeing, with DMF as solvent, was used to dye (immobilize) TPP onto medium weight, plain weave 100% cotton fabric. Under room temperature, cotton fabric was soaked in 0.5 mg/ml TPP−DMF dyeing bath, which was placed on an Innova 2000 platform shaker (New Brunswick Scientific, Edison, NJ) at 100 rpm shaking rate. After 4-h dyeing, the cotton fabric was soaked in 1 M NaCl at 4 °C overnight and then soaked in 50% ethanol for 30 min. Deionized water was used to rinse NaCl and ethanol from the TPP dyed cotton fabric. In the investigation of diazinon detection by TPP dyed cotton fabric, diazinon was dissolved in methanol. A plastic clip was used to hold the fabric flat against the inner wall of the standard 3-ml glass cuvette (Starna Cells, Atascadero, CA), in which 2.6 ml of 50 mM pH7 sodium phosphate buffer was added. Absorption spectra in the presence and absence of diazinon were collected at room temperature using Cary 300 UV−visible spectrophotometer with an internal mounted 70 mm diffuse integrating sphere. Difference spectra and peak positions in difference spectra were obtained using Grams/AI, and linear regression was performed using PSI-Plot.

3. Results and discussion

In DMF solution, TPP has a peak absorbance at 417 nm (Fig. 2, trace 1). After diazinon was added to the solution, the spectrum slightly shifted to a shorter wavelength (Fig. 2,

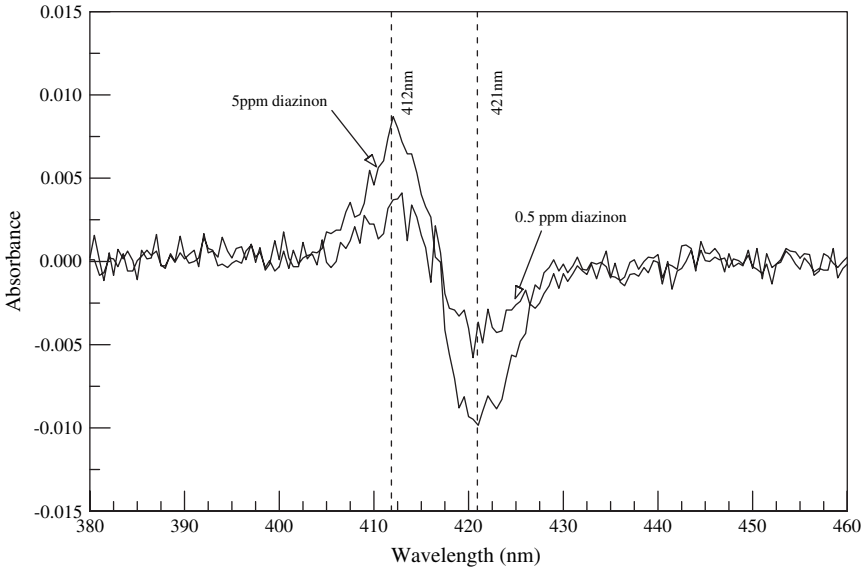


Fig. 3. Difference spectra (TPP + diazinon) − TPP in DMF solution after adding 0.5 ppm and 5 ppm diazinon.

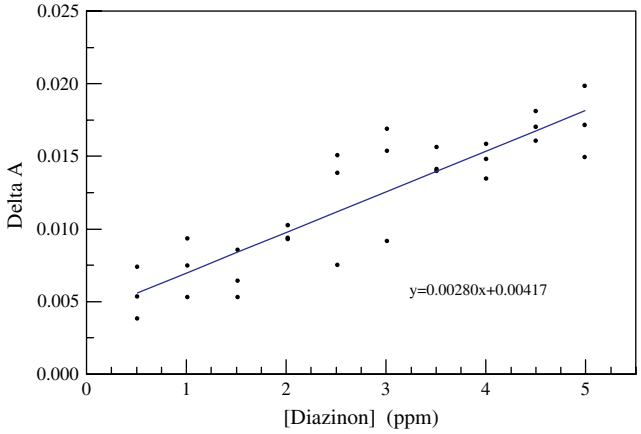


Fig. 4. The linear relationship between absorbance difference (ΔA between 412 nm and 421 nm) in difference spectra and diazinon concentration in TPP–DMF solution.

trace 2). This shift was more obvious if we take a spectrum subtraction (TPP + diazinon) − TPP (Fig. 2, trace 3). Fig. 2 (trace 3) shows that the difference spectrum (TPP + diazinon) − TPP has a peak at 412 nm and a trough at 421 nm. The difference spectra of (TPP + diazinon) − TPP after adding 0.5 ppm and 5 ppm diazinon are shown in Fig. 3. From Fig. 3, we can see that peak and trough positions are always in 412 nm and 421 nm, respectively, in difference spectra no matter what the diazinon concentration is. Using difference spectrum, a small spectrum shift can be detected. Fig. 3 shows a larger difference between peak (412 nm) and trough (421 nm) absorbance (ΔA) in difference spectrum and is also associated with a higher diazinon concentration. Fig. 4 shows a linear relationship between ΔA in difference spectra and the diazinon concentration, which suggests the formation of TPP–diazinon complex. The linear relationship is $\Delta A = 0.00280 \times [\text{diazinon}] + 0.00417$ ($R^2 = 0.792$). In the detection protocol, diazinon concentration can be determined from this linear relationship. In solution, diazinon can be detected at 0.5 ppm level by TPP.

After soaking in 1 M NaCl and 50% ethanol, there are substantial (visible) amounts of TPP remaining on cotton fabrics, which suggests good dyeing (immobilization) capability of TPP. TPP dyed cotton fabric has a peak absorbance of 0.30 at 420 nm (Fig. 5, trace 1). Similar to liquid phase diazinon detection in DMF solution, after adding diazinon, TPP dyed cotton fabrics have a slight spectral shift to a shorter wavelength (Fig. 5, trace 2), which is also more obvious in difference spectrum (Fig. 5, trace 3). Fig. 5 (trace 3) shows that the difference spectrum (TPP + diazinon) − TPP has a peak at 415 nm and a trough at 430 nm. The 415 nm peak and 430 nm trough in difference spectrum are not observed when pure methanol instead of diazinon–methanol solution is added. This suggests that peak and trough at these two wavelength positions are associated with the interaction between TPP and diazinon. This will assure selective detection of diazinon by TPP. The difference between peak (415 nm) and trough (430 nm) absorbance (ΔA) in difference spectrum is proportional to diazinon concentration. Fig. 6 shows a linear relationship between ΔA and diazinon concentration. The linear relationship is $\Delta A = 0.000195 \times [\text{diazinon}] - 0.000543$ ($R^2 = 0.807$). In solid state detection protocol, diazinon can be detected at 11 ppm level by TPP dyed cotton fabric.

Without the presence of enzyme AChE or OPH, TPP has the capability to detect diazinon due to the spectral shift associated with the interaction between these two compounds. However, in the absence of enzymes, TPP does not have the superior diazinon detection sensitivity of enzyme-based sensors, which could detect 45 ppt diazinon in the presence of AChE [11] and 800 ppt diazinon in the presence of OPH [9]. TPP has an improved diazinon detection sensitivity in solution (0.5 ppm) as compared with being dyed on cotton fabric (11 ppm). The immobilization of TPP on the fabric surface reduces the sensitivity of TPP when interacting with diazinon.

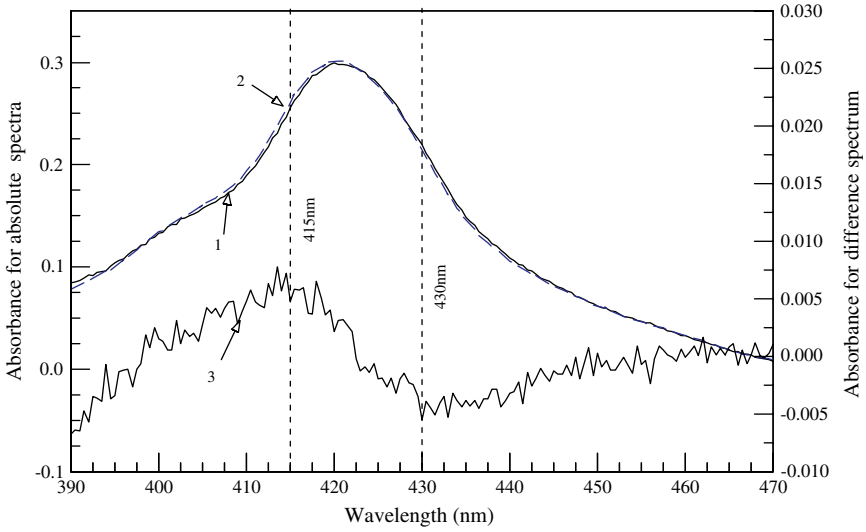


Fig. 5. The absorbance spectrum of TPP dyed cotton fabric in the absence and presence of diazinon at pH 7. The substraction of the absolute spectrum of TPP (trace 1) from the absolute spectrum of TPP + diazinon (trace 2, dashed line) gives the difference spectrum (TPP + diazinon) − TPP (trace 3).

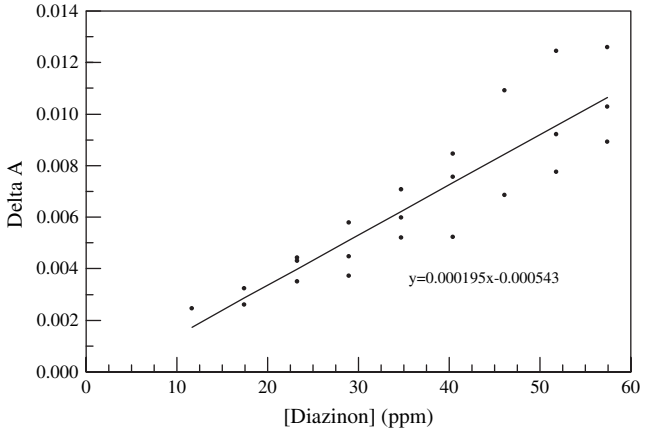


Fig. 6. The linear relationship between absorbance distance (ΔA between 415 nm and 430 nm) in difference spectra and diazinon concentration for TPP-dyed cotton fabric.

Compared with the smooth glass sensing surface in enzyme-based sensors [9,11], the irregular surface of the cotton fabric also makes the spectral shift of porphyrin when interacting with diazinon less detectable. The fact that no enzymes are involved in this detection protocol ensures that diazinon sensors based on this protocol can be stored and used in a wide range of temperature and pH conditions.

4. Conclusion

In DMF solution and dyed cotton fabric, the absorbance spectra of porphyrin TPP shift when bound with OP compound diazinon. These spectral shifts are more obvious in the difference spectra with a peak and a trough in specific wavelength positions. This property can be used in diazinon detection. Diazinon concentration can be determined from the linear relationship between ΔA in the difference spectrum and the diazinon concentration. Since no enzyme is used in this detection protocol, the diazinon sensor based on this protocol can be stored and used in a wide range of pH and temperature environments. With a textile fabric as the detecting surface, this study implies that an OP detection smart textile (textile as OP sensor platform) can be developed after other sensor components such as light source and light detector are embedded into the textile.

Acknowledgments

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