Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Stilbene-Based Fluorescent Sensor for Detection of Organophosphorus Warfare Nerve Agents

Robinson Anandakathir[a]; Umaprasana Ojha[b]; Earl T. Ada[c]; Rudolf Faust[b]; Jayant Kumar[ad]
[a] Center for Advanced Materials, University of Massachusetts Lowell, Lowell, MA [b] Department of Chemistry, University of Massachusetts Lowell, Lowell, MA [c] Campus Materials Characterization Laboratory, University of Massachusetts Lowell, Lowell, MA [d] Department of Physics, University of Massachusetts Lowell, Lowell, MA

To cite this Article Anandakathir, Robinson , Ojha, Umaprasana , Ada, Earl T. , Faust, Rudolf and Kumar, Jayant(2009) 'Stilbene-Based Fluorescent Sensor for Detection of Organophosphorus Warfare Nerve Agents', Journal of Macromolecular Science, Part A, 46: 12, 1217 — 1222
To link to this Article: DOI: 10.1080/10601320903340325
URL: http://dx.doi.org/10.1080/10601320903340325

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2009) **46**, 1217–1222

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601320903340325

Taylor & Francis
Taylor & Francis Group

Stilbene-Based Fluorescent Sensor for Detection of Organophosphorus Warfare Nerve Agents

ROBINSON ANANDAKATHIR[1], UMAPRASANA OJHA[2], EARL T. ADA[3], RUDOLF FAUST[2] and JAYANT KUMAR[1,4,*]

[1]*Center for Advanced Materials, University of Massachusetts Lowell, Lowell, MA 01854*
[2]*Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854*
[3]*Campus Materials Characterization Laboratory University of Massachusetts Lowell, Lowell, MA 01854*
[4]*Department of Physics University of Massachusetts Lowell, Lowell, MA 01854*

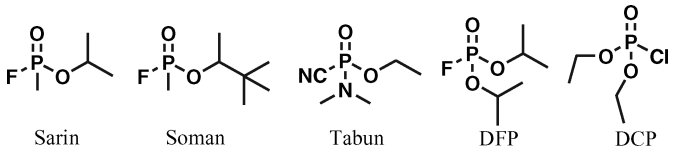
In this paper, we report the synthesis of stilbene-based fluorophore, 3,4-dihydroxy-4′-aminostilbene (DHAS) for the detection of chemical warfare agents such as organophosphorus nerve gases. DHAS was characterized by various spectroscopic methods and grafted on to electrospun nanofibers. The interaction of DHAS with nerve agents simulant, diethyl chlorophosphate (DCP) was investigated in solution and vapor phase by fluorescence spectroscopy.

Keywords: Nerve agents, stilbene, fluorescent sensor, nanofibers, DCP

1 Introduction

Besides biological and nuclear threats, chemical warfare nerve agents are some of the most feared weapons of mass destruction. Nerve agents including Sarin, Tabun and Soman are colorless, volatile and highly toxic organophosphorous (OP) compounds. The toxicity of nerve agents is mainly due to their ability to inhibit the action of serine proteases, most notably acetylcholinesterase, a critical enzyme in central nervous system. The deactivation of the enzyme results due to the reaction between the hydroxyl group at the active site of enzyme and the nerve agents, leading to organ failure and eventual death of victims. The ease of production and acute toxicity of these nerve agents emphasize the need to develop reliable, affordable and real-time systems for their detection. Several methods developed for detection of OP nerve agents include surface acoustic wave (SAW) devices, enzymatic assays, electrochemistry, interferometry, fluorescence spectrometry and gas chromatography-mass spectrometry (1–6).

The OP nerve agents are very reactive and could be easily attacked by nucleophilic reagents like water or hydroxyl groups. Based on this concept, several fluorescent-based sensors have been developed and used for the detection of OP nerve agents. In earlier studies, compounds such as



diisopropylfluorophosphate (DFP) and diethylcholorphosphate (DCP) have been used as typical model compounds. In basic medium, phenolic hydroxyl group possesses the ability to attack the phosphorus atom of nerve agents to form the corresponding ester. The reactivity of the phenolic hydroxyl group with organophosphorus compound was increased by the introduction of another hydroxyl group in the ortho position (7, 8). A fluorescent stilbene-based compound containing two hydroxyl groups was designed and synthesized to take advantage of the enhanced reactivity. In this paper, we report the synthesis, characterization and photophysical properties of 3,4 dihydroxy-4′-aminostilbene and its interaction with nerve agent simulant (DCP) after functionalizing with nanofibers.

2 Experimental

2.1 Materials

3,4-dihydroxy benzaldehyde, 4-nitrophenyl acetic acid, acetic anhydride, zinc dust, 4-fluorobenzenesulfonyl chloride, 1,12-diisocyanatododecane, 4-dimethylaminopyridine (DMAP), dicholorophosphate and all the

*Address correspondence to: Jayant Kumar, Center for Advanced Materials, University of Massachusetts Lowell, Lowell, MA 01854. E-mail: jayant_kumar@uml.edu

solvents were purchased from Sigma-Aldrich, USA. Poly(hydroxybutylmethacrylate) (PHBMA) was purchased from Scientific Polymer Products, Inc., NY, and used as received.

2.2 Characterization

The ^{1}H-NMR spectrum was recorded on a Bruker Instrument Inc., DPX 500 spectrometer at 500 MHz using TMS as the internal standard. Infrared spectra were recorded from neat samples using Nicolet 4700 Fourier transform infrared (FT-IR) spectrometer. UV-Visible spectra were recorded using a Perkin-Elmer Lambda 9 spectrophotometer. Fluorescence spectra were measured on a Perkin-ElmerLS-55 spectrophotometer.

2.2.1. *Synthesis of 3,4-diacetoxy-4′-nitrostilbene(4)*

3,4-Dihydroxy-4′-nitrostilbene (**3**) was prepared by a known method (9, 10). 3,4-dihydroxybenzaldehyde (1.38 g, 10.0 mmol), 4-nitrophenylacetic acid (1.81 g, 10.0 mmol) and piperidine (0.43 g, 5.2 mmol) were heated at 120–130°C for 3 h under nitrogen. The resulting 3,4-Dihydroxy-4′-nitrostilbene was dissolved in pyridine (10 ml) and acetic anhydride (5 ml) was added slowly at 0°C. After stirring at room temperature for a period of 3 h, the reaction mixture was diluted with dichloromethane (60 ml) and washed several times with dilute hydrochloric acid (40 ml each) and water (30 ml each). Then organic layer was dried over magnesium sulfate and concentrated in *vacuo* to afford the crude brown solid product. It was purified by column chromatography using silica gel as absorbent and hexane–EtOAc/3:1 as the eluent. A chromatographic fraction corresponding to $R_f = 0.4$ on TLC (SiO_2, hexane–EtOAc/3:1 as the eluent) was isolated to afford the synthesized 3,4-diacetoxy-4′-nitrostilbene, as yellow solid in 76% yield (2.62 g), m.pt. 161–163°C.

^{1}H-NMR (500 MHz, $CDCl_3$, δ) 8.20 (d, $J = 8$ Hz, 2H, $H_{3'}$ and $H_{5'}$), 7.58 (d, $J = 8$ Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.37 (d, $J = 8.5$ Hz, 1H, H_5), 7.24–7.14 (m, 3H, Ph-CH =, H_2 and H_6), 7.05 (d, $J = 16.5$ Hz, 1H, Ph-CH =), 2.30 (s, 3H, CH_3-CO), 2.27 (s, 3H, CH_3-CO).

FT-IR (KBr) υ_{max} 3117, 3080, 2913, 2823, 1755, 1586, 1502, 1367, 1325, 1204, 1178, 1106, 981, 846, 750 cm^{-1}.

2.2.2. *Synthesis of 3,4-Dihydroxy-4′-aminostilbene (5)*

3,4-diacetoxy-4′-nitrostilbene (1.08 g, 3.0 mmol) was refluxed with zinc (0.92 g, 14.0 mmol) in the mixture of 2M HCl (10 ml) and methanol (70 ml) for 12 h. Then, the reaction mixture was filtered and washed with 95% methanol. The filtrate was treated with activated charcoal and filtered. The reaction mixture was kept in refrigerator after removing methanol for 12 h and centrifuged to afford 3,4-dihydroxy-4′-aminostilbene (**5**) as the brown solid product in 67% yield (0.440 g), m.pt. 228–230°C.

^{1}H-NMR (500 MHz, $CDCl_3$,δ) 8.87 (s, 1H, Ph-OH), 8.75 (s, 1H, Ph-OH).7.20 (d, $J = 8.0$ Hz, 2H, $H_{2'}$ and $H_{6'}$), 6.90 (s, 1H, H_2), 6.78–6.65 (m, 4H, Ph-CH = CH-, H_5 and H_6), 6.54 (d, $J = 8.0$ Hz, 2H, $H_{3'}$ and $H_{5'}$), 5.16 (s, 2H, -NH).

FT-IR (KBr) υ_{max} 3480, 3371, 2929, 1596, 1511, 1437, 1403, 1295, 1244, 1174, 1102, 962, 845, 820, 782 cm^{-1}.

2.3 Electrospinning

The PHBMA solution was prepared in THF and electrospun using electrospinning apparatus, which consists of a DC high power source (Gamma High Voltage Research Inc., FL), glass pipette and metal electrode wires. The polymer solution was in a glass pipette, into which the charged electrode of DC power supply was inserted. A grounded aluminum foil was used as the counter electrode for collecting fibers on a glass substrate at a distance of 15 cm from the tip of the pipette. The appropriate driving voltage was 20–25 kV. For the cross-linking of PHBMA nanofibers, 1,12-diisocyanatododecane (3%) was added to the polymer solution. After electrospinning the nanofibers, the sample was heated at 80°C in the vacuum for 1 h to complete the crosslinking.

2.4 Functionalization of PHBMA Nanofibers with 3,4-Dihydroxy-4′-aminostilbene

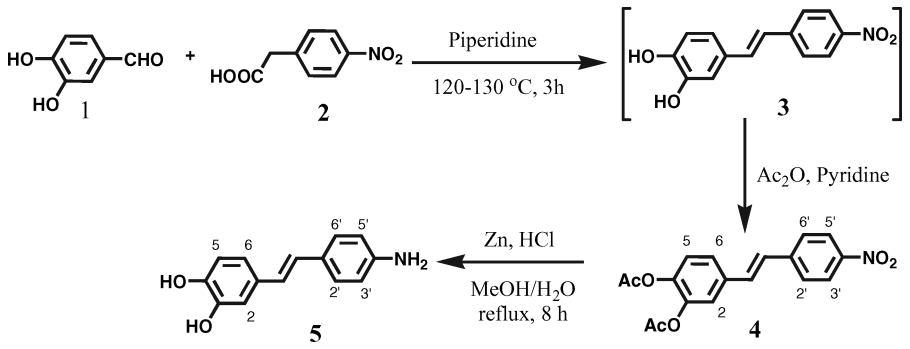
The crosslinked PHBMA nanofibers on a glass substrate were dipped in a standard solution of acetonitrile (6 ml), DMAP (0.030 g, 0.245 mmol), fosyl chloride (0.050 g, 0.257 mmol) and pyridine (1 ml) at room temperature. The nanofibers were removed after 10 min and washed with distilled water, followed by methanol. The fosylated nanofibers were reacted with DHAS (0.010 g in 10 ml THF) at 45°C for 12 h and washed with THF and dried under vacuum.

2.5 X-ray Photoelectron Spectroscopy (XPS)

Surface analysis of the nanofibers was performed with XPS using a Vacuum Generators ESCALAB MKII instrument under ultrahigh vacuum ($<10^{-9}$ Torr) using Mg Kα X-rays (1253.6 eV). The photoelectrons were energy-analyzed with a concentric hemispherical analyzer in fixed analyzer transmission mode using a pass energy of 20 eV. All specimens for surface analysis were prepared onto steel alloy disks. Data processing and curve fitting were performed using Avantage software. XPS spectra were recorded at a 90° takeoff angle.

3 Results and Discussion

We have synthesized stilbene based fluorophore using two-step procedure as illustrated in Scheme 1. 3,4-Diacetoxy-4′-nitrostilbene (**4**) was synthesized from 3,4-dihydroxy benzaldehyde (**1**) and 4-nitrophenyl acetic acid (**2**) by heating with piperidine, and then hydroxyl groups were protected with acetyl groups using acetic anhydride and pyridine.



Sch. 1. Synthesis of 3,4-dihydroxy-4′-aminostilbene.

Then diacetyl protected nitrostilbene (**4**) was reacted with zinc in the presence of hydrochloric acid to yield the desired stilbene fluorophore (3,4 dihydroxy-4′-aminostilbene, **5**). The product was characterized by ^{1}H NMR and FT-IR spectroscopy and the photophysical properties were studied using UV-Visible spectroscopy and fluorescence spectroscopy techniques.

The UV-Visible and emission spectra of the DHAS in tetrahydrofuran are shown in Figures 1 and 2. DHAS showed an absorption maximum (λ_{max}) at 340 nm and showed the fluorescence with maximum emission wavelength of 390 nm upon excitation at 340 nm in THF.

^{1}H-NMR spectra of stilbene derivates and the starting materials are shown in Figure 3. In the case of diacetoxy derivative (**4**), acetyl protons were detected at δ2.27 ppm (Figure 3d) while the hydroxyl protons of the 3,4-dihydroxy-4′-nitrostilbene (**5**) appeared as two singlet peaks at δ8.87 and 8.75 ppm (Fig. 3e). The amino protons of **5** were observed as broad singlet peak at δ5.16 ppm (Fig. 3e).

To understand the behavior of stilbene based system in the presence of nerve gas simulant (Dicholorophosphate, DCP), first a series of fluorescence experiments were carried out in THF (Fig. 4). The fluorescence of DHAS (1×10^{-6} M) solution in THF was quenched by the addition of DCP (10 mM). The quenching increased with time for the duration of our measurements.

The quenching behavior of DHAS was then studied with a different concentration of DCP. The results showed that the fluorescence intensity of DHAS (2.8×10^{-6} M) decreased gradually with the increase of DCP concentration (Fig. 5). The quenching of the emission band might be due to the interaction of fluorophore and analyte molecule through hydrogen bonding (8).

The fluorescence quenching sensitivity in solution can be quantified through measurements of the Stern-Volmer constant (K_{SV}) described in Equation 1.

$$I / I_0 = 1 + K_{SV} [Q] \quad (1)$$

I_0 and I represent the intensities of fluorescence in the absence and in the presence of the DCP respectively. Q represents the concentration of the quencher, DCP molecules. A linear relationship between the concentration of DCP and I_0/I was obtained. The Stern-Volmer constant (K_{SV}) of the DHAS was calculated from the slope of the plot (Fig. 6), and it was found to be 2.59×10^2 M^{-1}.

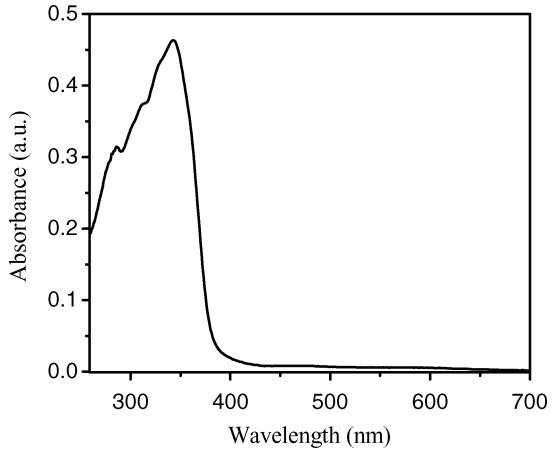


Fig. 1. UV-Vis absorption spectrum of the 3, 4 dihydroxy-4′-aminostilbene (**5**).

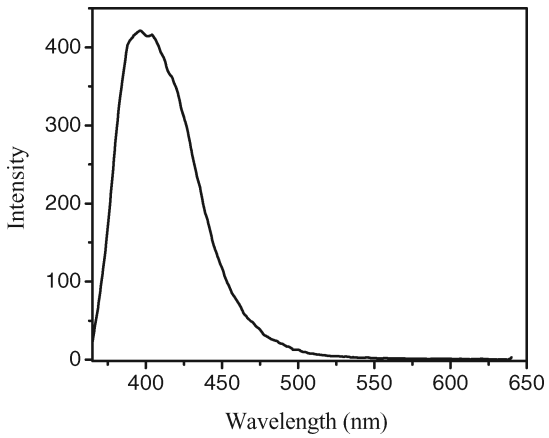


Fig. 2. Fluorescence emission spectrum of the 3, 4 dihydroxy-4′-aminostilbene (**5**).

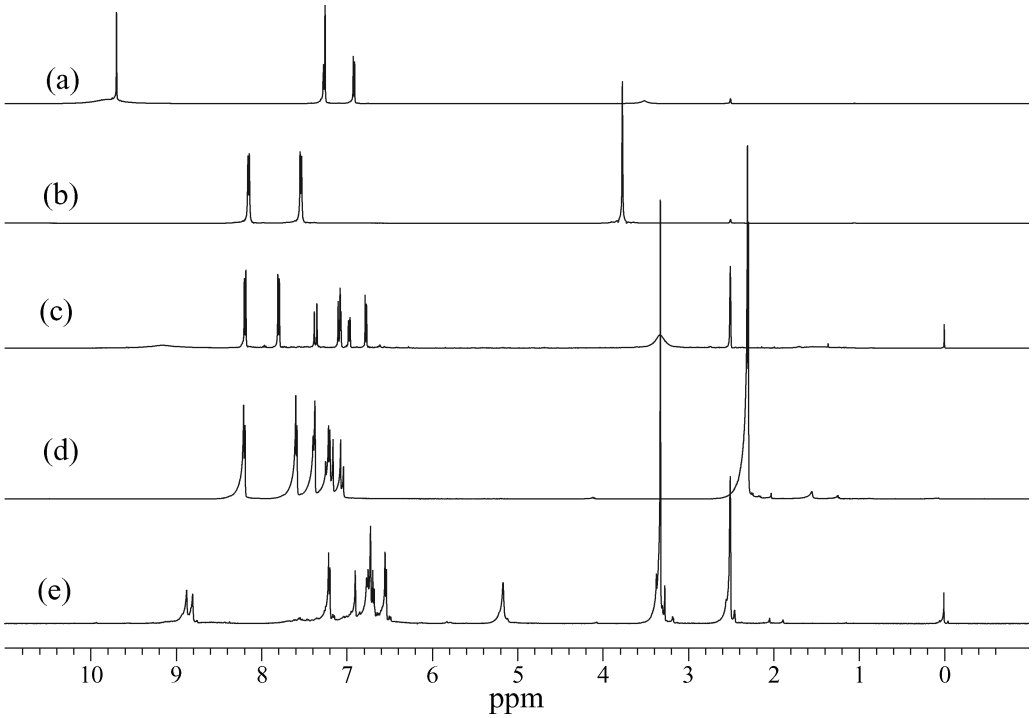


Fig. 3. ^{1}H-NMR spectra of (a) 3,4-dihydroxybenzadehyde in $CDCl_3$, (b) 4-nitrophenyl acetic acid in $CDCl_3$, (c) 3,4 dihydroxy-4′-nitrostilbene in DMSO-d_6, (d) 3,4 diacetoxy-4′-nitrostilbene $CDCl_3$ and (e) 3,4 dihydroxy-4′-aminostilbene in DMSO-d_6.

For the sensitive vapor phase detection of DCP, the stilbene based fluorophore DHAS was covalently attached to polymer (PHBMA, **6**) nanofibers. Nanofibers expected to provide large surface area and increase the interaction of fluorophores with analyte molecules. Electrospinning was used to produce polymer nanofibers by applying a large static electrical charge to a polymer solution. The functionalization of the PHBMA nanofibers with DHAS was first attempted. However the PHBMA fibers by themselves were also soluble in THF. Hence, PHBMA fibers were first crosslinked using 1,12-diisocyanatododecane and fibers became insoluble in THF. The cross-linked fibers were fosylated with 4-fluorobenzenesulfonyl chloride (fosyl chloride) in acetonitrile in the presence of 4-dimethylamino pyridine (11, 12). Then, the fosylated nanofibers, **7** were reacted with DHAS in THF at 45°C for 12 h to give DHAS functionalized nanofibers, **8** (Scheme 2).

Each stage of activation on the surface of nanofibers was investigated using X-ray photoelectron spectroscopy. XPS

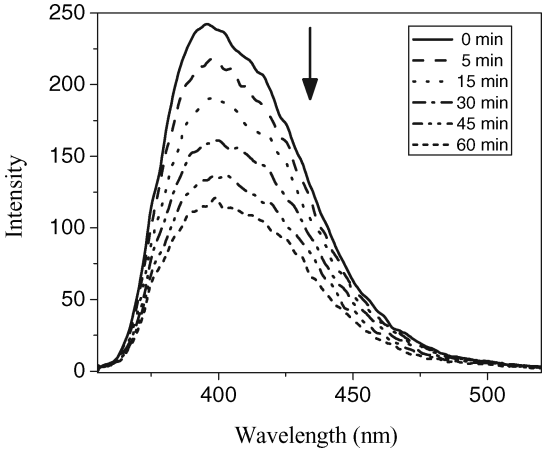


Fig. 4. Fluorescence spectra of 3,4-dihydroxy-4′-aminostilbene in THF at different times on the addition of 10 mM of DCP.

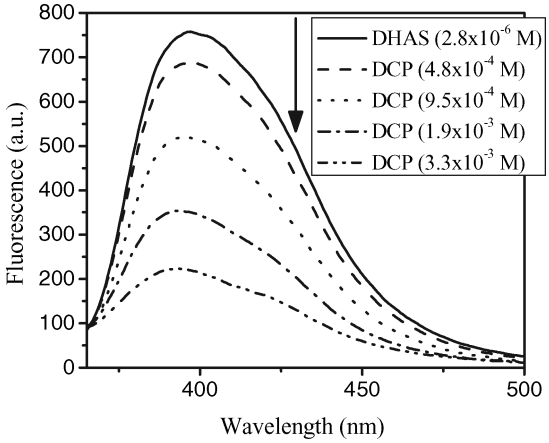
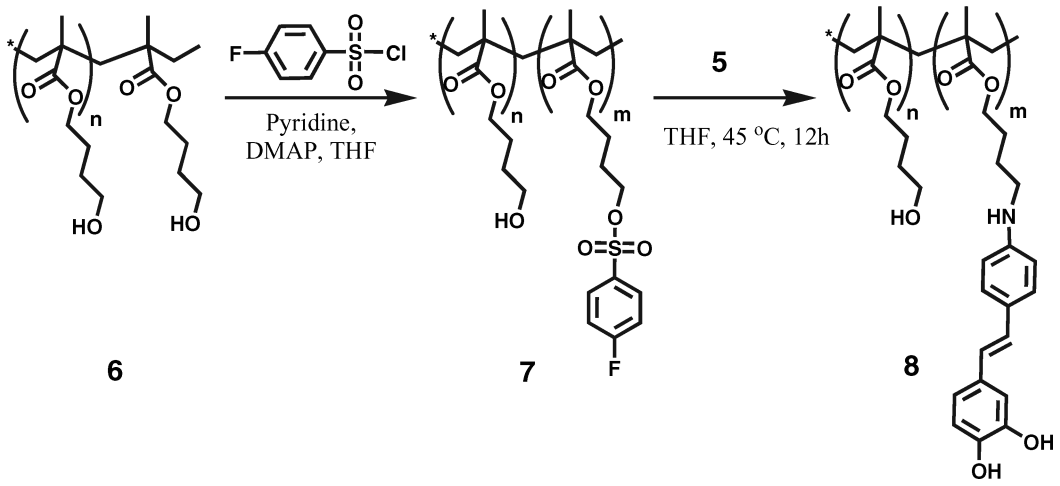


Fig. 5. Fluorescence quenching of 3,4-dihydroxy-4′-aminostilbene in THF on the addition of different concentration of DCP.



Sch. 2. Synthesis of DHAS functionalized PHBMA Fibers.

analysis of fosylated nanofibers shows S2p peak at an (uncorrected) binding energy of 170.4 eV, which corresponds to the sulfonate group of the 4-fluorobenzenesulfonyl moiety. DHAS functionalized nanofibers shows N 1s peak at a higher binding energy (400.1 eV), which corresponds to the amine group in DHAS (Fig. 7). This confirms the attachment of DHAS on the PHBMA nanofibers.

The fluorophore functionalized nanofibers showed a fluorescence intensity maximum at 390 nm upon excitation at 330 nm. After exposing to DCP vapors, the intensity of emission band at 390 nm was quenched with the increase of time (10% in 10 min and 40% in 40 min). Full quenching can be achieved after a few hours of exposure while a detectable decrease of fluorescence was observed in minutes. The vapor sensitivity of functionalized nanofibers towards DCP vapor is about a few parts per thousand

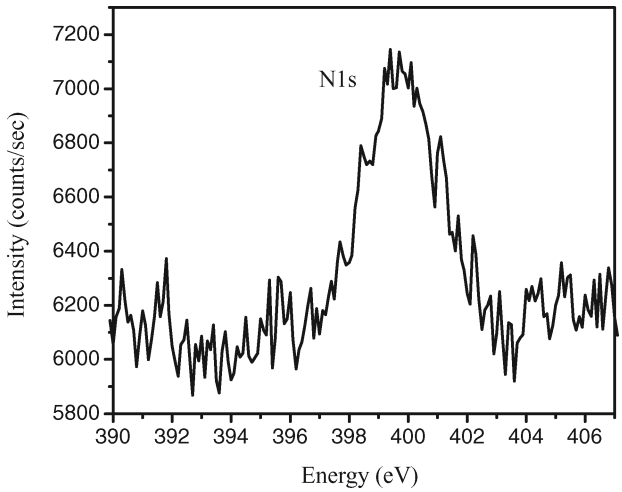


Fig. 7. N1s XPS spectra of DHAS functionalized on PHBMA nanofibers.

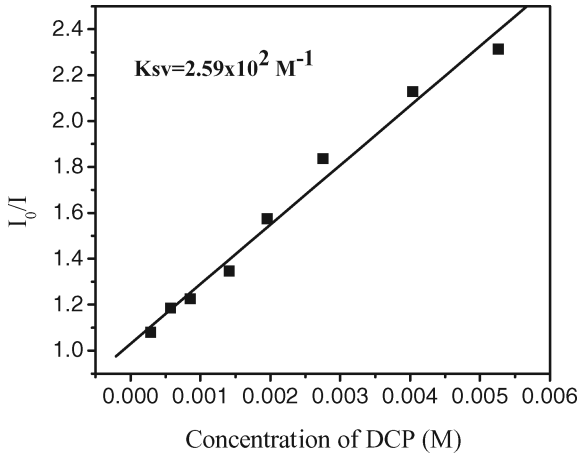


Fig. 6. Stern-Volmer plot of DHAS (2.8×10^{-6} M solution) as a function of the DCP concentration.

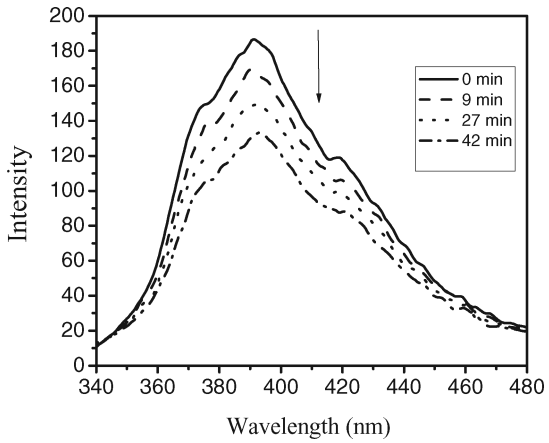


Fig. 8. Response of DHAS functionalized PHBMA Fibers on exposure to DCP vapor at room temperature (25°C).

and it can be improved considerably by more sophisticated methods.

4 Conclusions

Stilbene-based fluorophore, 3, 4-dihydroxy-4′-aminostilbene was designed, synthesized and characterized for detection of chemical warfare agents such as organophosphorus nerve gases. DHAS was covalently attached to the cross-linked nanofibers, which were fabricated by electro-spinning technique, and its specific interaction with nerve agents was investigated. The experimental observation shows quenching of fluorescence in stilbene chrompohores upon interaction with a simulant (DCP) of nerve gases.

Acknowledgments

Financial support by Army Research Laboratory [Award # V911NF-07–2-0081] and National Science Foundation [Award # NSF ECCS-0601602] are gratefully acknowledged.

References

1. Burnworth, M., Rowan, S.J. and Weder, C. (2007) *Chem. Eur. J.*, 13, 7828–7836.
2. Royo, S., Manez, R.M., Sancenon, F., Costero, A.M, Parra, M. and Gil, S. (2007) *Chem. Comm*., 4839–4847.
3. Giordano, B.C. and Collins, G.E. (2007) *Curr. Org. Chem.*, 11, 255–265.
4. Dale, T.J. and Rebek, J. Jr. (2006) *J. Am. Chem. Soc*., 128, 4500–4501.
5. Zhang, S.W. and Swager, T.M. (2003) *J. Am.Chem. Soc.*, 125, 3420–3421.
6. Viveros, L., Paliwal, S., McCrae, D., Wild, J. and Simonian, A. (2006) *Sens. Actuators, B*, 115, 150–157.
7. Jandorf, B.J., Wagner-Jauregg, T., O'Neill, J.J. and Stolberg, M.A. (1952) *J. Amer. Chem. Soc.*, 74, 1521–1523.
8. Grate, J.W. (2008) *Chem. Rev.*, 108, 726–745.
9. Cullinane, N.M. (1923) *J. Chem. Soc. Trans*., 123, 2053–2060.
10. Lee, J.Y., Bang, H.B., Park, E.J., Lee, W.J., Rhee, B.K. And Lee, S.M. (2004) *Polym. Int*., 53, 1838–1844.
11. Yu-An Chang, Y.A., Gee, A., Smith, A. and Lake, W. (1992) *Bioconjugate Chem*., 3, 200–202.
12. Ojha, U., Feng, D., Chandekar, A., Whitten, J.E. and Faust, R. (2009) *Langmuir*, 25, 6319–6327.