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Rapid and Sensitive Strip-based Quick Test for Nerve Agents Tabun, Sarin and Soman Using BODIPY-modified Silica Materials

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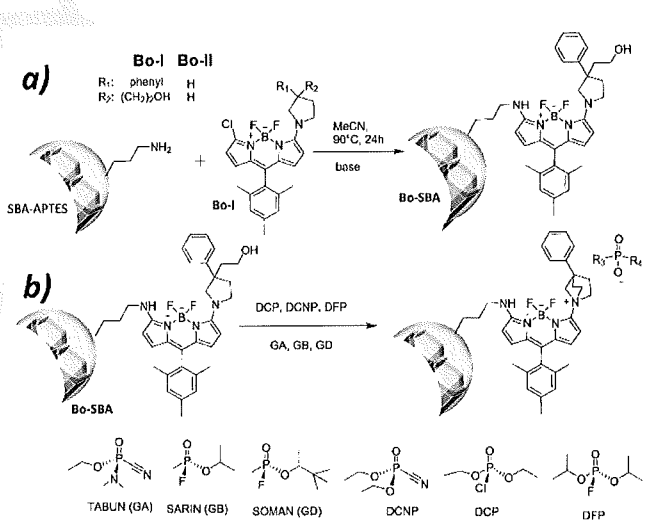
Abstract: The development of test strips that in combination with a portable fluorescence reader or digital camera can rapidly and selectively detect chemical warfare agents such as GA, GB and GD and their simulants in the gas phase is described. The strips contain spots of a hybrid sensing material consisting of a fluorescent BODIPY probe covalently anchored into the channels of mesoporous SBA silica microparticles. The fluorescence quenching response to nerve gases allows for the sensitive detection of these chemical warfare agents in the µg m^{-3} range.

The organophosphate nerve agents Tabun (GA), Sarin (GB) and Soman (GD) are among the most toxic chemical warfare agents (CWAs) known today.[1] The compounds are chemically similar to organophosphate pesticides. They are colourless liquids that are volatile at ambient temperatures, and exert their biological effect by irreversibly inactivating acetylcholinesterase (AChE) enzymes of the human nerve system.[2] Since the first chlorine gas attacks in 1915, in WW I, which killed and injured several 1000 soldiers, poisonous gases are banned as weapons of mass destruction, their release presenting a serious threat to humanity.[3] However, since more than 20 years CWAs have still been used in offensive ways against civilian population by terrorists such as in Tokyo in 1995 or in Syria in 2013.[4] Moreover, after very recent attacks in Paris in November 2015 the French government authorized the distribution of atropine, a competitive inverse agonist to organophosphates, to expand security measures and potentially counter any follow-up incidents utilizing nerve gases.[5] Whether public safety, the safety of soldiers or specialists working in CWA disposal is concerned, the need for simple and rapid detection methods that are applicable at site by untrained personnel is increasingly important. Chemical sensors are perhaps the most versatile approach in this regard with respect to sensitivity, selectivity and miniaturization. During recent years, a number of optochemical detection schemes have thus been conceived, mostly relying on oxime or hydroxyl groups as the reactive entity.[6] These chemical groups mimic the reaction of AChE with the nerve agents.[1,2] Despite the elegance of many of these studies, most of them report on the detection in organic solvents rather than in the gas phase and commonly employ so-called nerve agent simulants (NASs) instead of GA, GB or GD.[7] Just a few examples directly report on the determination of nerve agents.[8]



Scheme 1. (a) Synthesis of sensing material **Bo-SBA** and (b) proposed sensing paradigm and chemical structures of chemical warfare agents GA, GB and GD as wells as the nerve agents simulants DCNP, DCP and DFP employed in this study.

Our development of a potent, simple and robust detection system for CWAs in the gas phase reported herein follows a different approach. First, we envisaged the formation of a bicyclic [2.2.1]-type ring system directly integrated into the chromophoric π system of a bright fluorescent probe of the BODIPY (boron–dipyrromethene) family, **Bo-I**, as the reaction product between CWAs and sensor material as proposed in Scheme 1. Second, the probe was covalently anchored into the pores of mesoporous silica of SBA-type (**Bo-SBA**, Scheme 1), to endow the system with stability and allow for facile implementation with supports.

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Bo-SBA was then spotted onto conventional test strips for use in gas phase sensing. Strips were chosen here because they guarantee fast interaction with the analyte, are cheap, easy to handle and potentially mass-producible. To test the performance of our material, we first carried out studies with the frequently employed NASs diethylchlorophosphate (DCP), diethylcyanophosphonate (DCNP) and diisopropylfluorophosphate (DFP) before turning to the actual CWAs GA, GB and GD, elucidating whether the small but apparent differences in chemical structure have an impact on sensitivity, selectivity or response time.

Bo-SBA was prepared in two-steps. First, extracted SBA-15 microparticles with a cylindrical morphology of 1 × 0.5 µm (l × d) were functionalized with 3-aminopropyltriethoxysilane (APTES) in acetonitrile (MeCN) to provide amino groups for subsequent anchoring on the materials' surface. Second, the obtained SBA-APTES material was suspended in a solution of MeCN containing **Bo-I** and an excess of *N,N*-diisopropylethylamine, and reacted for 24 h at 90 °C to graft the probe to the material's pore walls (Scheme 1; see Section 2, Supporting Information (Supporting Information) for more details). The amount of BODIPY units on the surface of **Bo-SBA** was determined to 0.081 mmol g^{-1}.

To assess the performance of **Bo-SBA** in a convenient manner in the laboratory and to get a general idea about its suitability for the intended application, the hybrid sensor particles were tested in solution in the presence of the NASs DCP, DFP and DCNP. The fluorescence of suspensions of **Bo-SBA** (0.05 g mL^{-1}) in MES buffer at pH 6 were thus measured in the presence of different amounts of NASs in 10 mm cells in a fluorometer, revealing an enhanced quenching of the fluorescence as the concentration of the NAS was increased (see the example of DCNP in Figure S4, Supporting Information). Support for the proposed mechanism, i.e., formation of a phosphate ester derivative followed by an intraannular cyclization (Scheme 1), was obtained with the help of material **Bo-SBA'**, containing **Bo-II** (Scheme 1) that does not possess a hydroxyl group and is thus unable to undergo the indication reaction. A comparison of the two materials is also depicted in Figure S4 from which the absence of a pronounced quenching effect for **Bo-SBA'** upon exposure to of up 0.5 mM DCNP is readily visible. A second series of experiments in solution with the NASs as well as the actual CWAs then revealed that the latter induce a similar modulation of the fluorescence properties of **Bo-SBA** (0.8 g mL^{-1}), see the photographs taken under a UV lamp (λ_{exc} = 365 nm) after 4 min of reaction in Figure S5, Supporting Information.

Encouraged by these results in solution we approached gas phase detection and prepared test strips by disposing several spots of a suspension of **Bo-SBA** on commercial silica (TLC) and nitrocellulose strips (Figure S6, Supporting Information). The former worked better in our case because they are more robust facilitating handling in many different environments; all results reported in the following were thus obtained with the siliceous support. The strips were exposed to an atmosphere containing high amounts of DCP, DCNP and DFP (1 g m^{-3}) at 25 °C in a closed container of 19 mL, the latter being heated for 2 min at 80 °C to generate the gas of the corresponding simulant. After cooling the containers for 5 min, the fluorescence of the strips was observed under a UV lamp and also measured with a flow assay reader. With the example of DCP, Figure 1a shows that a change of the colour of the spots from pink to slightly yellowish-grey was observed in normal room light in all cases. When irradiated with UV light the colour pattern includes a change from orange to grey (DCP), green (DFP) and yellow (DCNP). In order to quantitatively estimate these changes, the fluorescence of the strips was measured with a fluorescence reader (λ_{exc} = 520 nm, λ_{em} = 625 nm) before and after exposure to the NAS. In all cases, a strong decrease of the signal was observed which is exemplarily shown in Figure 1b for DFP. The quenching was quantitatively analysed as the ratio between the intensity areas after and before exposure (I/I_0), yielding a quenching efficiency of 59%, 68% and 74% for DCNP, DFP and DCP, respectively.

Based on these preliminary results, we studied the response over time in more detail. Several strips were left in atmospheres containing 70 mg m^{-3} of DNCP, DCP or DFP and the fluorescence of the strips was measured at certain time intervals with the flow assay reader. In all cases a decrease of the fluorescence intensity was observed with time (Figure 2a with the example of DCP). As a control, the same experiment was performed in the absence of NASs in normal air atmosphere. Figure 2b shows the corresponding time traces. Whereas the fluorescence remains virtually constant in the absence of NAS, a decrease of the fluorescence in the time regime of minutes was observed in the presence of the simulants. The reactivity of the strips toward the NASs follows the order DCNP < DFP ≅ DCP.

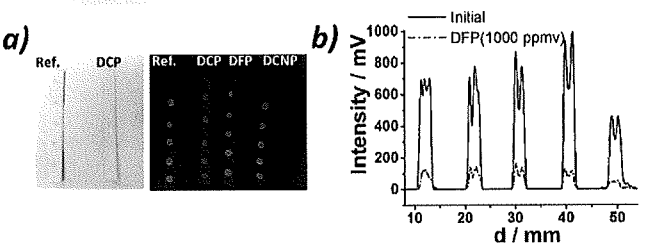

Figure 1. (a) Colour and fluorescence (under UV lamp with λ_{exc} = 365 nm) pattern of spots of **Bo-SBA** on strips before (denoted "Ref.") and after exposure to NASs DFP, DCP and DCNP. (b) Signal obtained with fluorescence reader (λ_{exc} = 520 nm, λ_{em} = 625 nm) from train of spots disposed on a strip before (solid line) and after (dotted line) leaving the strip in a saturated atmosphere (1 g m^{-3}) of DFP; d = distance on strip relative to the terminal end of the strip holder.

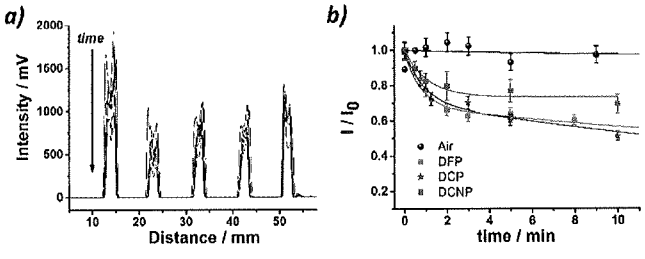

Figure 2. (a) Intensities of the signals of the strips containing **Bo-SBA** obtained with the fluorescence reader (λ_{exc} = 520 nm, λ_{em} = 625 nm) from a series of deposited spots before and after leaving the strip in an atmosphere of DCP (70 mg m^{-3}) for certain time intervals. (b) Fluorescence quenching kinetics recorded in a similar manner for DCP, DCNP and DFP at 25 °C; reference labelled "Air".

A good candidate material for sensing nerve agents in the gas phase must respond rapidly, sensitively and selectively. We thus decided to take 7 min, at which the fluorescence was quenched by ca. 80% (using 1 g m^{-3} NASs), as a preliminary benchmark to assess the sensitivity of the response. Following the same procedure as described above, several closed vessels containing a strip and 5 µL of different concentrations of NASs in hexane were left for 2 min at 80 °C in order to generate the corresponding gas. After 5 min at room temperature, the vessels were opened and the fluorescence of the strips was measured. As can be seen in Figure 3, the sensitivity for the simulants follows the order DCNP << DFP < DCP. The same experiment was also carried out with HCl and H_2O, to evaluate possible degradation or cross-sensitivity due to the presence of these species. Obviously, only high amounts of HCl are able to produce a certain decrease in fluorescence of a maximum of 20%. The dose-effect curves were then fitted to a four-parameter logistic function to determine the limits of detection (LOD). The LODs were found to be 123.5, 21.3 and 0.085 µg m^{-3} for DCNP, DFP and DCP, respectively, calculated by subtracting three times the standard deviation (3σ definition) from the maximum signal observed. It is important to note that these LODs found for the simulants are below the values attributed to nerve agents for causing adverse effects on human health, all lying higher than 1 mg m^{-3} (Table S3),[9] and are also below the LODs reported by other authors,[10] most of these previous works simply checking the efficiency of the sensor in an atmosphere saturated with NASs.[11]

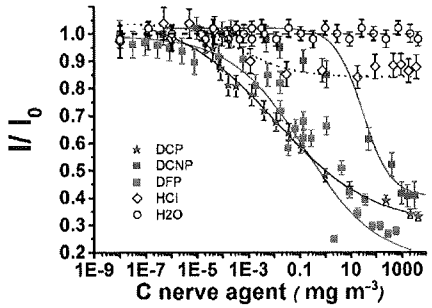

Figure 3. Fluorescence quenching of **Bo-SBA** on strips left in atmospheres of DCNP, DFP and DCP as well as HCl and H_2O at different concentrations. Data were obtained with the fluorescence reader after 7 min of exposure.

Regarding the potential interference of acids, time-resolved fluorescence studies were carried out for **Bo-SBA** deposited on slides after exposure to atmospheres containing NASs as well as HCl at concentrations of 1 g m^{-3} at which the responses are virtually complete (Figure 3). Despite the fact that the anchored BODIPY dyes show a non-exponential decay, which is typical for dyes confined in such narrow pore systems,[12] the average lifetime of **Bo-SBA** of 2.2 ns is reduced to 1.9, 1.6 and 1.2 ns in the presence of DCNP, DCP and DFP, respectively, yet remains virtually unchanged (2.1 ns) in HCl atmosphere. Protonation thus seems to appear non-selectively and mainly at the silica surface of the scaffold material, the altered polarity and electrostatic landscape of which might have an entirely physical effect on the fluorescence, inducing for instance only slight solvatokinetic modulations.

Encouraged by these results for the nerve agent simulants, we approached the actual CWAs Soman (GA), Sarin (GB), and Tabun (GD). First, we studied the reaction kinetics of the assay in the presence of the nerve agents, following the same procedure as for the simulants, i.e., exposing the test strips to an atmosphere containing 0.5 g m^{-3} of GA, GB and GD. As can be seen in Figure 4, **Bo-SBA** also responded to the nerve agents with a pronounced quenching of the fluorescence. Control experiments with 0.5 g m^{-3} DCNP, DCP and DFP revealed that the performance of **Bo-SBA** against the CWAs is comparable to that against DCNP, i.e., only slightly inferior compared with DFP and DCP. However, it is important to note that after ca. 5 min, fluorescence quenching is at 50%. When fitting the fluorescence changes vs. time to a first-order kinetic reaction model, assuming that the rate depends only on concentration, rate constants as well as half-lifetimes of k = 0.2–0.7 min^{-1} and $t_{1/2}$ = 1–4 min were determined, following the order $k_{GB} < k_{GD} < k_{DCNP} \cong k_{GA} < k_{DCP} < k_{DFP}$ (Table S2). This comparative investigation leads to the conclusion that the CWAs are only slightly less reactive than the NASs and that our approach is also suitable for the real targets.

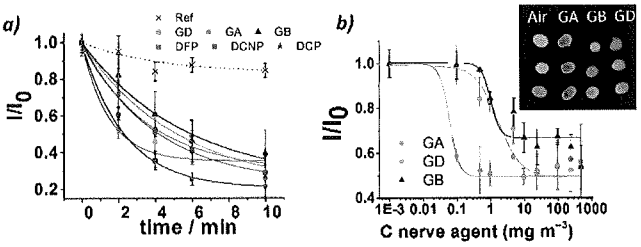

Figure 4. (a) Fluorescence quenching kinetics recorded with the fluorescence reader (λ_{exc} = 520 nm, λ_{em} = 625 nm) for **Bo-SBA**-containing test strips before and after exposure to an atmosphere of 0.5 g m^{-3} of CWA and NAS at 25 °C. (b) Fluorescence quenching of **Bo-SBA** on strips left in atmospheres of GA, GD and GB at different concentrations. Data were obtained with the fluorescence reader after 4 min of exposure. Inset: Colorimetric changes under UV lamp (λ_{exc} = 365 nm) in absence and presence of GA, GB and GD (0.5 g m^{-3}) after 4 min of reaction.

Having established the general suitability, the sensitivity of the assay was assessed. Since the kinetic profiles of CWAs are very similar and $t_{1/2} \leq 4$ min, we ran concentration series for GA, GB and GD in analogy to the respective tests for the NASs mentioned above, measuring the response after 4 min of exposure. Figure 4b illustrates that the fluorescence of the **Bo-SBA**-containing strips showed again a dose-response behaviour for these analytes, the reactivity following the order of GA >> GD > GB. Using the same procedure as described before for the simulants, LODs of 42, 560 and 832 µg m^{-3} were determined for the nerve agents GA, GD and GB, respectively. These values lie below the toxicity values established for these highly toxic compounds. Since already CWA concentrations of 1-3 mg min m^{-3} are known to cause miosis and higher concentrations lead to severe incapacity or death (see overview in Table S3)[13] yet our test strips are able to detect changes in the µg m^{-3} range, the strips

qualify as useful tools for the sensitive detection of nerve agents in acute scenarios.

With regard to realistic scenarios, however, the use of a fluorescence reader is disadvantageous. In a first step to simplification, we thus attempted an RGB analysis of the spectroscopic response as recorded after taking a photograph of the strips under UV illumination. Several strip assays were thus repeated with the NASs, photographs taken with a normal digital camera and the histograms of the three-dimensional RGB colour-space extracted. As depicted in Figure S8, the histograms of the red and blue RGB coordinates showed strong displacements of 20–30 units in the presence of DCP. DFP and DCNP at 1 g m^{-3} in the atmosphere. Taking the mean coordinate number of the histograms as a measure we were able to extract quantitative information.

Following the same procedure, the concentration effect of GA, GD and GB in the gas phase was evaluated by taking photographs after 4 min of exposure and extracting the relevant RGB data for analysis. Again, we referenced these results against similar tests for DCP, DCNP and DFP (Figures S9, S10). Table 1 lists the results of these experiments. As can be seen, the results of the blue RGB coordinate and the electronic reader agree rather well. It is obvious that the much simpler optical detection scheme and data treatment yields comparable LODs of 0.2–1 mg m^{-3} for CWAs and NASs as the electronic reader, showing now even the highest sensitivity for GA.

Table 1. Limits of detection (mg m^{-3}) determined for the nerve agents GA, GB, GD and the simulants DCP, DFP and DCNP by analysing either the blue or red RGB coordinates of photographs taken under the UV lamp or the fluorescence intensities obtained from the electronic reader.

Target	LOD (electronic reader)	LOD (blue RGB coordinate)	LOD (red RGB coordinate)
GA	0.042 ± 0.002	0.061 ± 0.005	0.13 ± 0.01
GB	0.56 ± 0.02	0.35 ± 0.01	0.70 ± 0.01
GD	0.83 ± 0.03	0.78 ± 0.06	0.75 ± 0.01
DFP	0.18 ± 0.02	0.21 ± 0.01	0.51 ± 0.03
DCP	0.73 ± 0.04	0.83 ± 0.04	1.00 ± 0.03
DCNP	0.94 ± 0.03	0.84 ± 0.04	0.97 ± 0.03

In conclusion, we have prepared test strips for nerve agent detection in the gas phase that contain physically embedded hybrid sensor microparticles consisting of a siliceous SBA-15 scaffold that carries reactive BODIPY probes covalently anchored to its inner pore walls. For probe design, we took advantage of the ability of a strategically positioned hydroxyl group to undergo an acylation reaction with phosph(on)ate substrates as proposed in Scheme 1, yielding a bicyclic [2.2.1]-cyclization product with quenched fluorescence. The choice of the chromophore allowed us to monitor the responses conveniently in the visible spectral range by either using a fluorescence reader or a digital camera. While assay optimization was carried out with the nerve agent simulants DCNP, DCP and DFP in the laboratory, experiments in a dedicated and controlled environment revealed that the performance of the sensing material is equally powerful when confronted with the real chemical warfare agents GB, GA and GD. The reactive strips reached detection limits in the µg m^{-3} range upon exposure to an atmosphere of the agents for a few minutes, meeting the sensitivity requirements for real applications. Furthermore, the excellent discrimination against acids avoids false positive responses. The studies presented here suggest that low-cost, portable, rapid and easy-to-handle tests for the detection of nerve agents might become available in the nearer future that can for instance be printed on the clothes of first responders or soldiers and might be read out by means of only a small suit- or helmet-mounted camera or a camera in connection with a simple, equally mounted excitation source such as an LED.

Experimental Section

Experimental details on the synthesis and characterization of the dyes, materials and hybrids as well as further information on the assays and relevant additional data are provided in the Supporting Information.

Acknowledgements

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Keywords: Nerve gases • Chemical warfare agents • Test strip analysis • Fluorescence • Hybrid sensor materials

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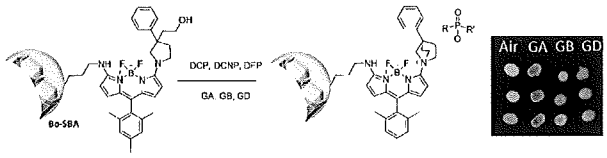

Test strips sense the toxic—Spots of a hybrid sensing material consisting of a fluorescent BODIPY probe covalently anchored into the channels of mesoporous SBA silica microparticles on a test strip detect chemical warfare agents by an optical response in the µg m^{-3} range.

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