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Chromogenic Detection of Nerve Agent Mimics by Mass Transport Control at the Surface of Bifunctionalized Silica Nanoparticles**

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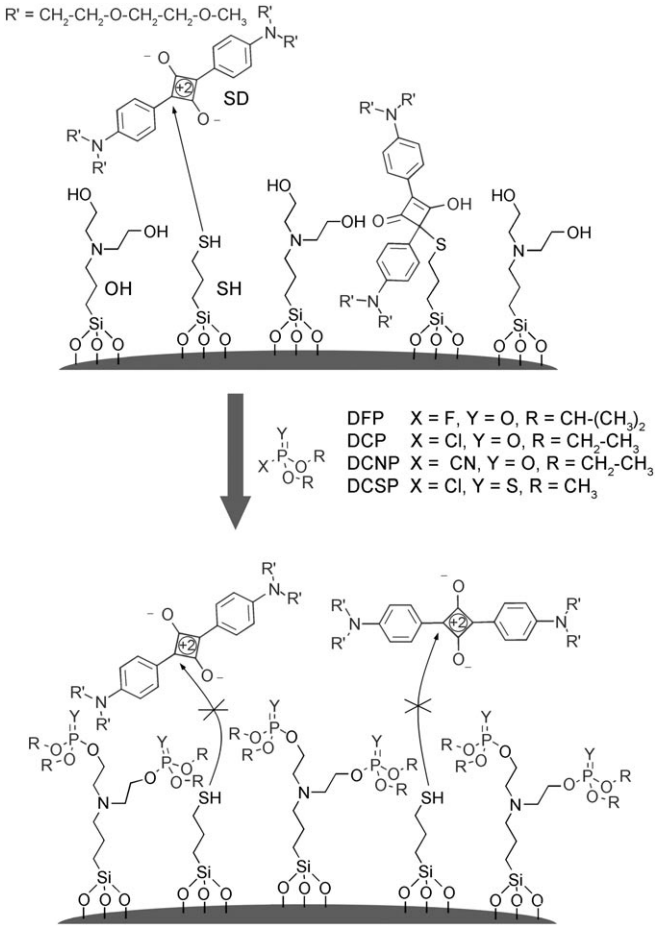
Chemical warfare (CW) agents are toxic chemicals that have been used in several terrorist attacks in recent years. Among CW species, nerve agents are probably the most dangerous; their high toxicity and facile synthesis underscores the need to detect these lethal compounds with quick, reliable procedures. Analytical methods based on enzymatic assays and physical measurements have generally been used to detect these hazards.[1] However, these protocols usually have limitations such as low selectivity, poor portability, and a certain level of complexity. In recent years, several chromogenic and fluorogenic sensors, and reagents for the detection of nerve agents have been described.[2] For instance, approaches that involve perborate-mediated oxidation of organophosphorus agents,[3] fluorescent probes based on polyethylene terephthalate (PET),[4] assays that use oximate-containing derivatives,[5] molecularly imprinted polymers,[6] nanoparticles,[7] carbon nanotubes,[8] porous silicon,[9] displacement-like procedures,[10] and cyclization reactions in push–pull chromophores[11] have been reported. Most of these protocols rely on changes in fluorescence properties, whereas few examples deal with color modulations. Colorimetric detection is particularly appealing because it uses low-cost, widely available instrumentation and allows assays to be detected with the naked eye. However, the development of selective, sensitive chromogenic probes for the detection of deadly chemical species is still rare.

Given our interest in the design of novel hybrid organic–inorganic materials as probes,[12] we focused on the preparation of a new optical test for the detection of nerve agent mimics based on nerve agent control of mass transport to the surface of functionalized silica nanoparticles. The chromogenic approach (Scheme 1) involves the use of silica nanoparticles that are functionalized with two different subunits—thiol groups (SH) and aliphatic alcohols (OH). The role of the



Scheme 1. Colorimetric protocol for the detection of nerve agent mimics. Top: bifunctionalized nanoparticles **N1** in the absence of mimics are able to react with squaraine (SD; bleaching of the solution occurs). Bottom: reaction of the mimics with the hydroxy groups (OH) inhibits the thiol–squaraine reaction (the solution remains blue).

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thiol moieties is to act as a reactive subunit towards a squaraine dye (SD). The reaction of the thiols with the central electron-deficient four-membered ring of the squaraine induces a loss of aromaticity, which is indicated by a bleaching of the blue squaraine solution.[13] Additionally, aliphatic alcohols are known to undergo acylation reactions with phosphonate substrates.[4,11]

The sensing protocol is based on the principle that the phosphorylation reaction of the OH groups with certain nerve agent mimics would inhibit the reaction between SH and SD, thus resulting in chromogenic signaling. Although both reactions are based on a nucleophilic attack, the thiol groups, which are more nucleophilic, are expected to react with the squaraine dye, whereas the larger size of the SH moiety would hamper its reaction with the electrophilic phosphorus atom of the mimics. Mass transport control to surfaces has been rarely explored in signaling approaches.[14–16]

Coated silica nanoparticles were prepared by using the trialkoxysilane derivatives 3-mercaptopropyltrimethoxysilane (MPTS) and 3-[bis-(2-hydroxyethyl)amino]propyltriethoxysilane (HPTS), following the procedure reported by Montalti et al.[17] In this synthetic protocol, silica nanoparticles were heated at 70 °C in a mixture of water/ethanol/acetic acid (1:2:1) in the presence of the coating subunits.

The obtained solid **N1-1** was characterized by standard procedures. The dialcohol and thiol contents were determined by elemental analysis and thermogravimetry, and amounted to 2.74 and 0.41 mmol g^{-1} SiO_2 respectively (see Table 1). One

Table 1: Thiol and dialcohol contents of the silica nanoparticles.

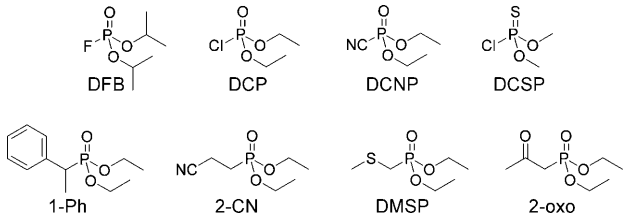
Compound	mmol thiol per g SiO_2	mmol alcohol per g SiO_2	Distance [Å][a]
N1-1	0.41	2.74	3.5
N1-2	0.06	0.44	8.3
N2	0.55	–	7.8

[a] Average distance between thiol and alcohol moieties.

important advantage of using silica nanoparticles in sensing protocols in solution is the possibility to prepare stable suspensions to monitor color changes, thus avoiding the need to filter, for instance, when larger silica particles are being used.

Signaling studies were carried out in the presence of nerve agent mimics DFP, DCP, DCNP, and DCSP, and in the presence of other phosphonates such as 2-CN, 1-Ph, DMSP, and 2-oxo (see Scheme 2). DFP, DCP, DCNP, and DCSP are organophosphates that have been widely used as nerve agent mimics as they display a similar reactivity to (but lack to the toxicity of) nerve agents such as Tabun, Sarin, and Soman.

In a preliminary experiment, 0.2 mL of a suspension of **N1-1** nanoparticles (3.7 g in 125 mL of 0.01 M pH 7.0 phosphate buffer) were mixed with 19.8 mL of acetonitrile. Then, 2.7 mL of this suspension were mixed with 0.15 mL of a solution of the squaraine dye in acetonitrile (1.0×10^{-4} M). Next, changes in the absorption band at 643 nm were monitored. The same protocols were subsequently carried



Scheme 2. Chemical structure of the nerve agent mimics and phosphonates used.

out with **N1-1** nanoparticles in the presence of DCP and DCNP.

As shown in Figure 1, the squaraine–thiol reaction is clearly controlled by addition of DFP, DCP, or DCNP. The squaraine–thiol reaction took place (there was a bleaching of

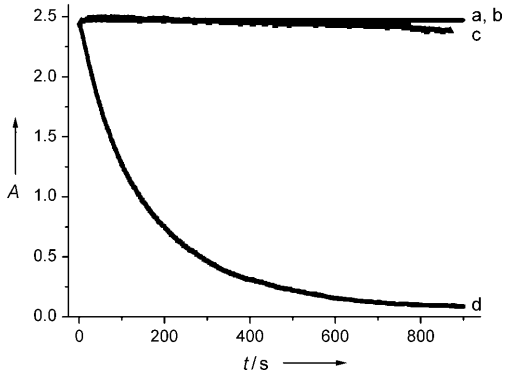


Figure 1. Absorbance at 643 nm (squaraine band) versus time for suspensions of **N1-1**. a) **N1-1** and SD in the presence of DFP, b) **N1-1** and SD in the presence of DCP, c) **N1-1** and SD in the presence of DCNP in acetonitrile, and d) only **N1-1** and SD.

the solution) in the absence of the nerve agent mimics, whereas the reaction was highly inhibited (the suspension remained blue) in the presence of DFP, DCP, or DCNP. Almost the same inhibition was observed with DCSP, whereas the presence of 2-CN, 1-Ph, DMSP, and 2-oxo resulted in similar reaction profiles to those observed for **N1-1** and SD alone (data not shown). The observed response is related to the reaction between nerve agent mimics and the hydroxy moieties anchored onto the silica nanoparticle. This reaction results in the formation of a dense monolayer of phosphate esters around the silica surface that limits the accessibility of the squaraine to the thiols. In order to corroborate the proposed mechanism, the hybrid nanoparticles **N2**, which were functionalized only with thiol moieties, were prepared. The addition of the squaraine dye to suspensions of **N2** in acetonitrile induced a rapid bleaching of the solution both in the absence and presence of the selected mimics.

In a typical assay, the effect of the guest concentration was studied. 0.2 mL of the aqueous suspension of **N1-1** nanoparticles (0.01 mol dm^{-3} in pH 7.0 phosphate buffer) were mixed with 19.8 mL of acetonitrile that contained a specific concentration of the guest. Then, 2.7 mL of this solution were mixed with 0.15 mL of a solution of the squaraine dye in

acetonitrile (1.0×10^{-4} M). After 10 minutes, the absorbance at 643 nm was measured. Figure 2 shows how the reaction between the nerve agent mimics and the hydroxy groups modulates the reactivity between the squaraine and the thiols, which is a function of the mimics concentration.

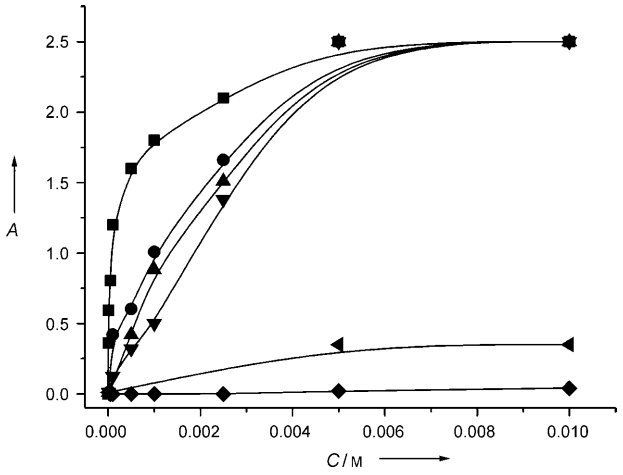


Figure 2. Absorbance at 643 nm (squaraine band) for suspensions of **N1-1**, SD, and varying concentrations of DFP (■), DCP (●), DCNP (▲), DCSP (▼), 2-CN (◀), and other species (◆; 1-Ph, DMSP, and 2-oxo) in acetonitrile.

The reaction between the thiol moieties on **N1-1** nanoparticles is severely hindered in the presence of DFP, DCP, DCNP, and DCSP, and the addition of 2-CN partially hampers the reaction at concentrations higher than 0.003 mol dm^{-3}. The presence of other mimics (1-Ph, 2-oxo, and DMSP) does not induce any effect, even at high concentrations (see Figure 2).

Figure 2 also shows that the chromogenic response for DFP clearly differs from that for DCP, DCNP, and DCSP, because a selective inhibition of the reaction between the thiol and the dye is observed at lower mimic concentrations. Figure 3 shows the absorbance of the squaraine dye at 643 nm in the presence of all the mimics, which were tested at a concentration of 1.0×10^{-5} mol dm^{-3}. Only DFP was able to partially inhibit the reaction of the squaraine dye with the thiol moieties, whereas the bleaching of the solution was complete in the presence of DCP, DCNP, DCSP, and other mimics tested.

To test the applicability of **N1-1** nanoparticles for the detection and quantification of nerve gases in real samples, it was confirmed that the sensing features of the **N1-1** support were retained in the presence of 5–10% water. Under these experimental conditions, the detection limit for DFP by fluorescence measurements ($\lambda_{exc}=643$ nm, $\lambda_{em}=670$ nm) was 5.0×10^{-6} M (1 ppb).

Although nerve agents could be detected by the remarkable color changes of **N1-1** nanoparticles, we observed that use of the solid **N1-2**, which contains fewer functional groups (see Table 1), was able to significantly improve the detection limit. The chromogenic response of suspensions of **N1-2** in acetonitrile clearly resembled the response of **N1-1**; that is,

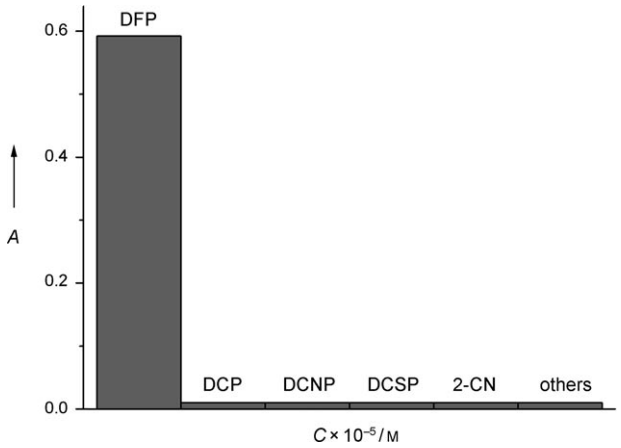


Figure 3. Absorbance at 643 nm (squaraine band) for 1.0×10^{-5} M suspensions of **N1-1**, SD, and DFP, DCP, DCNP, DCSP, 2-CN, and other guests (1-Ph, DMSP, and 2-oxo) in acetonitrile.

the presence of nerve agent mimics inhibited the reaction between SD and thiol moieties. Moreover, the **N1-2** suspensions that contained 5–10% water responded to nerve agent mimics with an improved detection limit of 1.0×10^{-8} M (2 ppt), as shown by fluorescence spectroscopy. The **N1-2** material also displays sensing features in the presence of 50% water, and still shows a remarkably low detection limit of 5.0×10^{-7} M for DFP (100 ppt; observed by fluorescence spectroscopy). This detection limit is close to those obtained by using molecularly imprinted polymers (50 ppt)[6c] or luminescent europium polymers (7–100 ppt),[6d] and is lower than the detection limits normally observed by using molecular-based fluorescent receptors for DFP (between 6 ppb and 15 ppm).[4,5]

To further probe the use of this system, the response of the **N1-2** suspensions in 1:1 acetonitrile/water were tested against alkylating and acylating agents. However, the presence of methyl sulfate, acetic anhydride, or acetyl chloride (at concentrations of 1.0×10^{-5} M) induced negligible changes in the kinetic profile of the reaction between the **N1-2** solid and SD. The response of the **N1-2** suspensions in buffered 1:1 acetonitrile/water was tested in the presence of the hydrolysis products such as HF, HCl, HCN, and H_2S (1.0×10^{-5} M), which are produced by nerve agent mimics; however, no interference was observed.

Finally, although a limitation of the two-step protocol must be stressed for certain applications, we were interested in checking the possibility of using the **N1-2** material for nerve agent detection in the vapor phase. The porous silica nanoparticles functionalized with the thiol and alcohol groups was applied onto a PET film to prepare a prototype of a dipstick assay. In a typical test, a functionalized stick was exposed to air containing 5 ppm DFP for 15 min. Then the dipstick was immersed in a solution of SD in acetonitrile. The SD solution was observed to remain blue (see Figure 4). In contrast, rapid SD bleaching was observed when performing the same experiment by using a dipstick that had not been in contact with vapors of the nerve agent mimic. This result indicates that DFP is able to react with the alcohol groups in the

Figure 4. Photograph of a 1×2 cm silica dipstick immersed in a solution of SD (2.6×10^{-6} M) in acetonitrile. The left-hand foil was previously exposed to air that contained 5 ppm DFP, whereas the right-hand foil was directly dipped into the SD solution.

dipstick, thus opening up the possible use of this or a similar solid as dosimeters for the evaluation of the accumulative exposure to nerve gas vapors.

In summary, we have developed an approach for the chromogenic sensing of nerve agent mimics by using silica nanoparticles that were functionalized with hydroxyl and thiol moieties. The presence of nerve agent mimics in suspensions of the hybrid silica nanoparticles controlled the access of squaraine to the surface of nanoparticles, hence resulting in a chromofluorogenic response. This procedure can be applied to mixed aqueous solutions and as a dosimeter for the detection of DFP in the vapor phase. The use of nanoparticles that can be easily functionalized with two or more individual groups that are able to react with dyes and nerve agents, and the possible use of a wide range of different units (i.e., dyes) for signaling makes this approach highly appealing and versatile for the design of chromofluorogenic probes for the detection of nerve agents.

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