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Selective colorimetric detection of Novichok agents with hydrazone chemosensors.

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KEYWORDS Novichok, hydrazone, colorimetry, chromogenic, detection, chemosensor, selectivity, nerve agents, 2,4-Dinitrophenylhydrazine

ABSTRACT: Recently, four new neurotoxic organophosphorus compounds classified as A-type agents were identified and added to the list of prohibited chemical warfare agents by the Organization for Prohibition of Chemical Weapons (OPCW). Called Novichok, they are organophosphorus derivatives and their rapid and selective detection remain a challenge. In the past years, several detection devices such as M8 or PDF1 colorimetric papers were developed to allow the rapid detection of these chemicals. It allows the rapid and selective detection of sarin, VX and sulphur mustard. A selective detection of Novichok will be a major advancement to prevent this new threat. In this regard, we developed a device specific to Novichok detection. Based on hydrazone derivatives, derived from 2,4-dinitrophenylhydrazine (2,4-DNPH), impregnated on a glass fiber substrate, it allows a rapid, selective and a naked eye colorimetric detection of Novichok agents. Due to its low cost, portability, high selectivity to Novichok and easy operation, they complete the detection range of M8 and PDF1 papers.

Developed during the World Wars¹, nerve agents are chemical warfare agents (CWAs). They are highly toxic organic chemicals². To prevent their development, production, stockpiling, the Organization for Prohibition of Chemical Weapons (OPCW) strives to fulfil the Chemical Weapons Convention³ (CWC). Even though the Convention aimed to strictly prohibit CWAs, their use has been a recurring issue and new substances were added to the list of prohibited compounds. Among these prohibited chemical weapons⁴ (fig. 1), we aim to detect preferentially organophosphorus nerve agents (OPs). OPs are inhibitors of acetylcholinesterase⁵. They used to be classified into two categories. G-type nerve agents, which include Sarin (GB), Cyclosarin (GF), Soman (GD) and Tabun (GA) are high vapour pressure liquids which implies an acute toxicity by inhalation. The V-series is the second family of organophosphorus nerve agents. Less volatiles but even more lethal, it includes VE, VG, VM and VX that exhibits a cutaneous contamination pathway. Due to their high toxicity, a direct exposure by inhalation or skin contact leads to death within few minutes.

Recently, four new neurotoxic organophosphorus compounds classified as A-type agents were identified and added to the list of prohibited nerve agents⁶ by the OPCW. Called Novichok, they are organophosphorus derivatives (Chart. 1). They exhibit different physico-chemical properties such as: a high solubility in water⁷, a very low rate of hydrolysis⁸, and a very low lethal dose⁷.

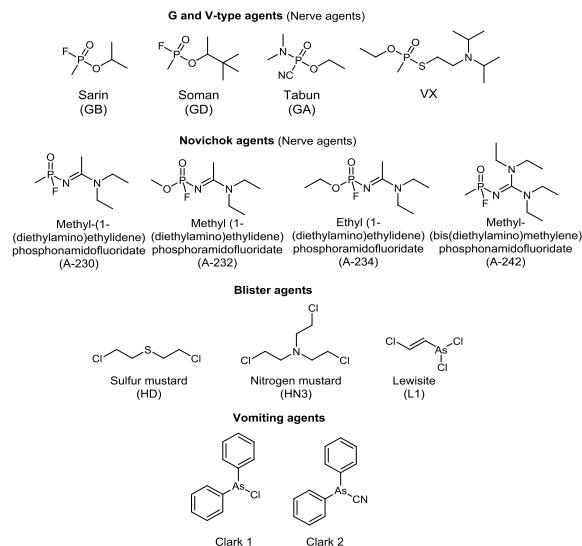


Chart 1. Structures of CWAs Sarin (GB), Soman (GD), Tabun (GA), VX, A-230, A-232, A-234, A-242, HD, HN₃, Lewisite (L1), Clark 1 and Clark 2

In order to better prevent this new potential threat, a selective detection of this new class of CWAs would be an asset. The chemical warfare detector paper PDF1 already provides a quick detection of the three main agents (GB, VX and HD)⁹. The identification of Novichok may complete this device to organize emergency and respond quickly in case of contamination.

In the present work, we propose a direct colorimetric method to specifically detect Novichok agents. Owing to the potential threat of these agents, it is critical to have an

effective detection system, which should detect specifically with ease and high rapidity this new type of OPs.

Many research groups are involved in CWAs detection. Different strategies can be used^{10,11}, including enzymatic^{12,13}, luminescence¹⁴⁻¹⁷ or colorimetric¹⁸⁻²¹ ways for the most represented. In addition, nanomaterials^{22,23} for sensing organophosphorus is currently another promising way. Using of probe array²⁴⁻²⁶ is also a possible way for discrimination of organophosphorus derivatives. Costero *et al.* have developed chromogenic^{24,27,28} or fluorogenic²⁹ probes to allow the detection of CWAs. Another relevant work for the CWAs detection by Pitschmann *et al.*³⁰ presented a simple way to selectively detect a wide range of CWAs with the combination of three chromogenic probes.

Our approach consists in using small organic molecules as chemosensors. A large screening of a chemical library of 1612 molecules were carried out to identify potential new chromogens for CWAs detection. In particular, we noticed that hydrazone compounds presented good preliminary results to detect CWAs (Chart 1.). Chemosensors used in this present work are hydrazones derived from 2,4-Dinitrophenyl hydrazine (2,4-DNPH). Impregnated in a specific support, these compounds can detect presence of these four new CWAs, the Novichok. The detection is visible to the naked eye and allows the discrimination from potential interfering compounds such as other OPs (G and V-series) and more generally CWAs. From the current research field so far, no similar detection of these new compounds have been reported.

Hydrazones are already widely used in many fields for their medicinal^{31,32} or optical properties³³. They are known for their solvatochromic³⁴, halochromic³⁵ and tautomerism properties³⁶. Hydrazone formation is characterized by a colour change, generally orange, which is used for carbonyl identification³⁷. They are known in coordination and complexation chemistry to be colorimetric sensors for diverse ions such as for example CN^- ³⁸, AcO^- ^{39,40}, F^- ⁴¹. Due to their specific molecular structure, they are also used as pH probes⁴². Moreover, due to their pH-responsive properties, they can be used for drug delivery systems⁴³. Their use for CWAs has been sparsely documented so far. Abdelrahman *et al.* report their use for CWAs simulant detection with the elaboration of a hydrazone-based supramolecular organogel⁴⁴.

Due to their acute toxicity and prohibition, only accredited person can handle CWAs. Therefore, literature is more documented for simulants detection than CWAs⁴⁵.

Herein after, we report that hydrazone derivatives can be chemosensors for specific detection of certain organophosphorus compounds. Deposited on glass fibers papers, hydrazones lead to a specific colorimetric response to 4 OPs, the Novichok compounds. These hydrazone chemosensors are composed of an electron-deficient 2,4-dinitrophenyl ring π -conjugated by an hydrazone link to an electron-rich aromatic part.

EXPERIMENTAL SECTION

Materials and General Methods. Hydrazone derivatives were prepared according to previously reported procedures^{46,47}. Carbonyl starting materials and solvents were purchased from Sigma Aldrich and used as received. 2,4-dinitrophenylhydrazine was provided from Chem-Lab (Belgium). Synthesis were followed by TLC on Merck aluminium foil with silica gel F₂₅₄. ¹H and ¹³C NMR spectra were recorded on a Bruker standard. Deuterated solvent, DMSO-d₆, was purchased from Sigma Aldrich. IR analyses were performed with a Bruker Vertex 70. UV-titration experiments were performed on an Agilent Cary 5000 UV-visible-NIR spectrophotometer with precision cells made from quartz, SUPRASIL 300, type No. 100-QX, ref. Z600164, with a 10 mm light path. A pH 110 VWR pH meter with combined glass electrode LiCl as electrolyte (SI Analytics 285092329, N 6480 Eth model) were used to conduct pH-measurements. High resolution mass spectrometry (HRMS) analysis were recorded on Thermo Fisher Q-Exactive by the Centre Régional de Mesures Physiques de l'Ouest (CRMPO).

General Procedure. 2,4-dinitrophenylhydrazine (1.3 eq) was solubilized in ethanol (0.1 M) at 50°C in presence of concentrated H₂SO₄ (3 eq). A solution of carbonyl (1 eq) in ethanol (1 M) was prepared and then added dropwise to the 2,4-DNPH solution. The reacting mixture was refluxed until complete conversion confirmed by TLC. For aldehyde reactants the reaction was practically instantaneous a stirring at 50°C was enough. Hydrazone products were partially insoluble. The product apparition can be confirmed by suspensions formation. The mixture was cooled to room temperature and 3/4 evaporated. The residue was stirred with a portion of water and a basic solution of 1 M NaOH. The hydrazone precipitate was filtered off and rinse twice with water. The crude solid was hot washed in the appropriate solvent, generally ethanol. The purified product was dried to give a colourful powder (yellow, orange or red) in good yield (70-98 %). The specific synthesis conditions and the characterization of the Hydrazone chemosensors 1 -11 is described in the Supporting Information.

Measurements of pKa. A fresh hydrazone solution was prepared at 0.0275 mM in DMSO. 100 mL of this hydrazone solution were stirred at room temperature and initial pH value was recorded with pH-meter. The solution was basified with addition of small amount (20 to 100 μL) of a 0.01 M NaOH solution. After each addition, pH value was recorded and a 3 mL of the stirring media were collected to record UV-visible spectra. The sample was reintroduced to the media and another amount of NaOH was added and continued until basic pH generally >13. pKa values were determinate via using Henderson and Hasselbalch equation. The exact determination has been more detailed in the Supporting Information.

Liquid sensing tests. Optical bottom plates, 96-well were used to conduct experiments. Each well were filled by 50 μL of a 0.5 mM solution of hydrazone. Each line corresponds to a different hydrazone. In each well of the first column, a volume of 2.7 μL of DMSO was added. This column was used as reference (Ref.) to compare experi-

tion to CWAs results. Other columns were exposed to CWAs, one column for one toxic: (from left to right) Sarin, Soman, Tabun, VX, A-230, A-232, A-234, A-242, HD, HN, Li, Clark 1 and Clark 2. CWAs were added with a multi-channel pipette. Each well containing hydrazone were completed with an additional volume of 2.67 μL of corresponding CWAs. Plates were scanned at 5 min and 1 h with an Epson Perfection V370 scanner after CWAs addition.

Sensibility tests. 11 solutions at different molarities of A-232 in DMSO were prepared: 0, 6.25, 12.5, 25, 62.5, 125, 250, 625, 1250, 2500 and 5000 mM. In an optical bottom plates 96-well, 50 μL of a 0.5 mM hydrazone solution were added. A selection of 8 hydrazones was tested (3, 4, 6, 7, 8, 9, 10, and 11). A line corresponds to one hydrazone. The concentration was fixed. The variable was the molar equivalent number of A-232 added. Novichok quantities were presented in terms of equivalents compared to hydrazones quantity (25 nmol per well). Equivalents were added in a range of 0.5 to 400 equivalents. One column corresponds to a fixed equivalent number: (from left to right) 0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 400 equivalents. In the first column, 2 μL of pure DMSO were added. This column was used as reference. In other columns 2 μL of different A-323 solution were added.

Interferent testing in liquid phase (DMSO). Two optical bottom plates 96-well were fulfilled with 50 μL of a 0.5 mM hydrazone solution in DMSO. A line corresponds to one hydrazone. To avoid contamination by adjacent columns two plates were used. A first one contained 6 interferents (water, H_2O_2 5%, acetic acid, 0.1 M HCl and gasoil) and a second for 6 another interferents (0.1 M NaOH aq, NaOCl 5%, 0.1 M KH_2PO_4 aq., ammoniac 5%, and trimethylamine). Aqueous solution were used at 5% and other interferents were added pure to hydrazones

Preparation of impregnated substrate. 1.5 μL of 10 mM hydrazone solution were impregnated on a glass fibers substrate. The substrate was completely dried before using.

Interferent testing on substrate. 1.5 μL of 10 mM hydrazone solution were impregnated on a glass fibers substrate. The substrate was completely dried before using. 1.5 to 1.7 μL of interferents (DMSO as reference, water, 0.1 M HCl aq, H_2O_2 5%, acetic acid, gasoil, 0.1 M NaOH aq, NaOCl 5%, 0.1 M KH_2PO_4 aq, ammoniac 5%, and trimethylamine) were pipetted and directly added on spotted hydrazones. Due to low hydrophilicity of the paper, aqueous solutions were diluted by ethanol 20% v/v. It allowed a good penetration of solutions in substrate and contact with hydrazones.

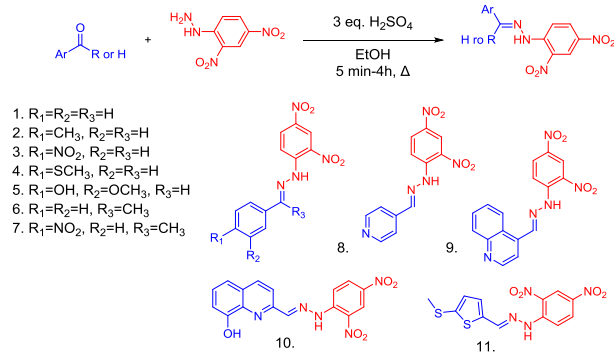
RESULTS AND DISCUSSION

Design and Synthesis of hydrazone chemosensors.

Hydrazone derivatives were synthesized by a one-step condensation reaction (Scheme 1) of the 2,4-dinitrophenylhydrazine (2,4-DNPH), with a ketone or an aldehyde in presence of 3 equivalents of concentrated

H_2SO_4 (96%). The reaction is widely used in literature and the hydrazone synthetic pathway was extracted from different researches^{46,47}. Hydrazone formation can be easily confirmed by the formation of a colourful suspensions. ^1H NMR spectra of synthesized compounds displayed a specific peak at δ 11-12 ppm typical of hydrazone bond. The molecular structure of hydrazone chemosensors were also confirmed by ^{13}C , FTIR, UV-Vis and HRMS spectral data. All information were detailed in Supporting Information.

Scheme 1. (Top) Synthesis of hydrazone; (bottom) synthesized structures: 1-6 derived from benzaldehydes, 7-8 derived from acetophenones, 9 derived from benzophenone, 10-13 derived from carboxaldehydes



11 hydrazones derivatives (Scheme 1) were synthesized to compare the influence of substituents on the chemosensors optical properties. All compounds are derived from 2,4-DNPH by changing the carbonyl starting material. The benzaldehyde derivative (1) was the reference structure. To compare substituents influence, several electron donating or withdrawing groups were chosen. The selected groups were $-\text{CH}_3$ (2), $-\text{NO}_2$ (3), $-\text{SCH}_3$ (4), and a combination of $-\text{OH}$ and $-\text{OCH}_3$ (5). In addition of benzaldehyde derivatives, acetophenone derivatives (6 & 7), two nitrogen heterocycles carboxaldehydes derivatives: pyridine (8) and quinoline (9 & 10) and a thiophene carboxaldehyde (11) were tested.

Structural variations even substitution of hydrazone chemosensors alter both chemical and physical properties. Structural changes can affect the stability of some electronic excited states even ground state and consequently coloration and reactivity for example. In this study, hydrazone chemosensors coloration was the main examined aspect. The starting materials nature influence the colour of resulting hydrazones derivatives. While the wavelength of the maximum absorption (λ_{max}) is 395 nm in DMSO for the reference benzaldehyde derivative (1), a more conjugated derivative like derivative (3) will have an higher λ_{max} ($\lambda_{\text{max}}=415$ nm). The presence of both electron-donating and electron-withdrawing substituent on benzaldehyde precursor leads to a bathochromic shift of the maximum absorption of hydrazones derivatives (1-5). The most significant increase was observed for the para-nitrobenzaldehyde based hydrazone (3) ($\lambda_{\text{max}}=415$ nm). It can be correlated to its strong electro-withdrawing properties. At the opposite, the $-\text{CH}_3$ para-substituent on hydrazone adduct (2) leads to a weaker bathochromic

shift ($\lambda_{\max}=399$ nm). These substituents are commonly called auxochromic groups. They extend the conjugation of the initial structure (benzaldehyde hydrazone derivative (1)) and causing a red shift. It is significant for p-NO₂ substituted chemosensors. Properly chosen substituents can modulate chemosensors response in terms of coloration and colorimetric response.

for Novichok detection, the two -NO₂ hydrazones lead to a more contrasted colorimetric response. Nitrogen heterocycle based hydrazone derivatives (9 & 10) caused a yellow to orange colour alteration, it was less contrasted than derivatives containing -NO₂. The colorimetric response in these cases (3 & 7) was yellow to brownish. As for Novichok sensing, the sensing of VX was more visual

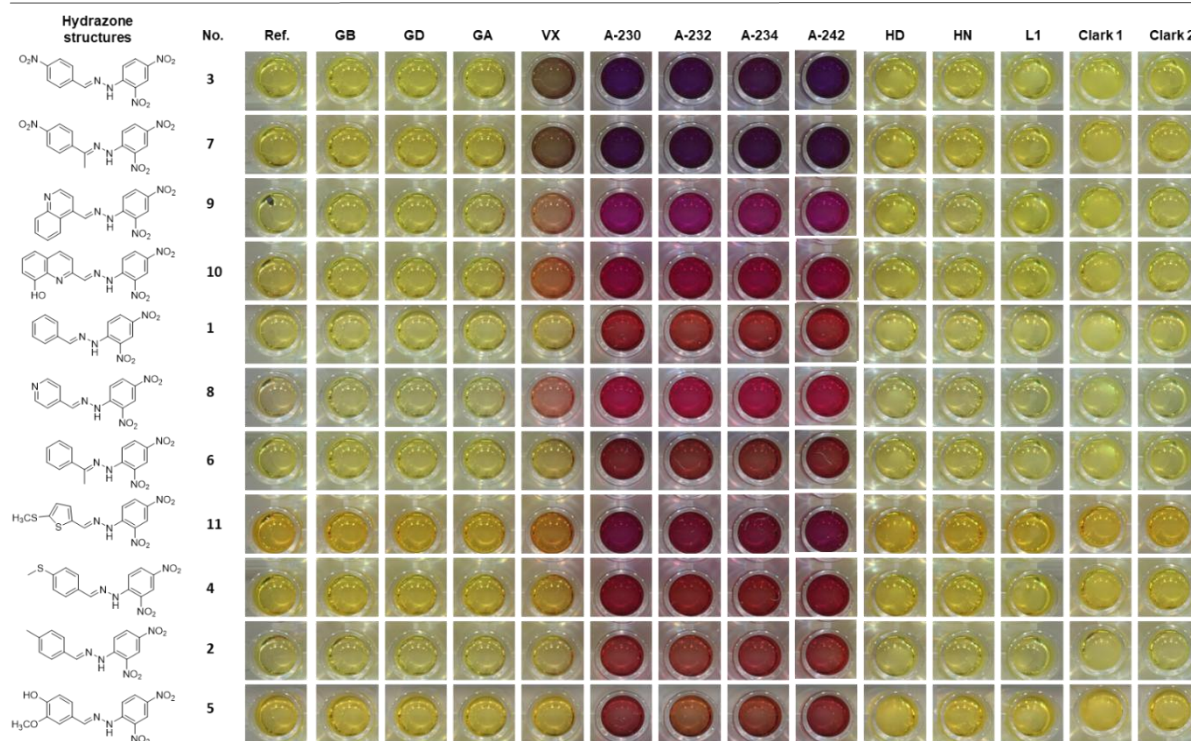


Figure 1. Hydrazones chemosensors at 0.5 mM in DMSO exposed to an excess of CWAs (400 molar equivalents)

Selectivity in liquid phase (DMSO). Colorimetric properties of Hydrazone chemosensors when exposed to Novichok were studied firstly in liquid phase. The sensing tests with CWAs were conducted in DMSO. Hydrazone chemosensors were dissolved in DMSO (at 10 mM) and exposed to different CWAs (400 equivalents). 13 CWAs were tested: Sarin, Soman, Tabun, A-230, A-232, A-234, A-242, Sulfur mustard HD, Nitrogen mustard HN₃, Lewisite L1, Clark 1 and Clark 2 (Chart. 1). 1.2 μ L of liquid toxics were added directly in solubilized hydrazones using a multichannel pipette. The results show a striking selectivity specifically for Novichok (Figure 1).

Hydrazones have a yellowish colour in DMSO. The addition of toxics leads to a colour alteration for all 4 tested Novichok. The resulting colour depends on hydrazone structure. The colour alteration can be reddish, magenta or purplish. The more contrasted alteration was obtained for hydrazones derived from carbonyls containing a -NO₂ group (3 & 7).

A colour alteration is also noticeable for VX with only 5 hydrazones (3, 7, 8, 9, and 10). The colour alteration is less contrasted than for Novichok. The resulting colour is generally brownish and less intense. The hydrazones (3, 7, 8, 9, and 10) were obtained from carbonyls containing either a -NO₂ group or a nitrogen heterocycle. As noticed

for hydrazone containing - NO₂.

For all other toxics tested (Sarin, Soman, Tabun, HD, HN₃, L1, Clark1 and Clark2) no colour alteration was noticed even after 1h. The liquid phase tests have shown a good selectivity for Novichok and in some cases for VX.

Hydrazones sensibility was also studied. A selection of 8 hydrazones were exposed to different quantities of the Novichok A-232. The added quantities of toxic were presented in terms of equivalents compared to hydrazones quantity (25 nmol per well). Equivalents were added in a range of 0 to 400 equivalents.

The colour of hydrazones gradually changed (fig. S4). The colour alteration was determined with the naked eye. The color change starts around 10 to 50 equivalents. Results were organized according to the better colour alteration for 10 equivalents. 4 derivatives (3, 7, 9 and 10) have presented a response around 10 equivalents and others (11, 4 and 6) around 20 to 50 equivalents. Only one tested derivative (10) have shown a weaker sensibility. The colorimetric response started at 50 equivalents and it was more contrasted at 100 equivalents.

Colorimetric properties. During our research, colorimetric properties were explored. Sensing studies were conducted in DMSO to avoid hydrazone precipitation due to their very low solubility in water. The UV-visible ab-

sorption spectra of hydrazones (1-11) were studied over the wavelength range of 200-800 nm.

The colour alteration was promoted by controlled addition of small amounts of a 0.01 M NaOH aqueous solution⁴⁸. After each addition, pH values were measured and UV-visible spectra were recorded. Changes in the absorption spectra are shown in figure 4 for the hydrazone (7). In DMSO, the pH was initially at 7.8. At first, hydrazone (7) solution in DMSO was yellow and the corresponding UV-visible band was measured at 395 nm. The colour was stable until pH reached 11.

A real colour alteration was observed from pH 11 to 12. Upon addition of sodium hydroxide, a diminution of the band at $\lambda_{\max}=415$ nm was observed while a new band at $\lambda_{\max}=576$ nm appeared. It was concomitant to the hydrogen deprotonation of the hydrazone bond (Figure 2.). At pH 11.5 and higher, the colour was darker for very basic conditions. The colour alteration was confirmed by the disappearance of the UV-visible band at 415 nm and the increase of the band at 576 nm. Finally, in all cases, hydrazones take a colour from purple to blue.

The colour change is due to a structure alteration induced by the deprotonation of the hydrazone moiety⁴⁹. This deprotonation promotes an electronic delocalization, which induces a more conjugated structure (Scheme 2.). On the absorption spectra, it leads to the formation of a new absorption band in the 490-580 nm region. The acid dissociation constant (pKa) of hydrazones were determined from their UV-visible absorption spectral behaviour (Table. 1).

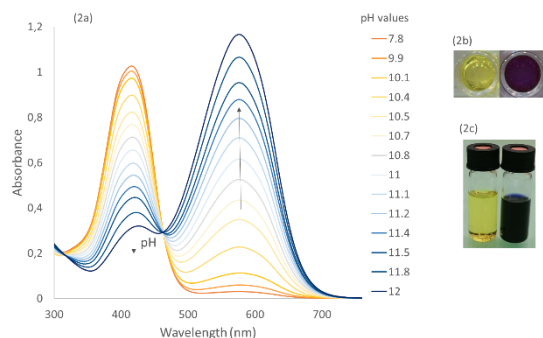
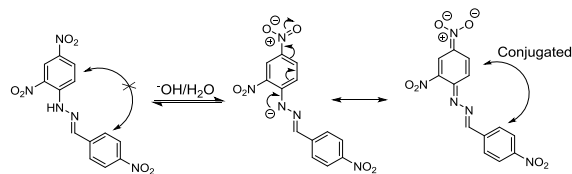


Figure 2. (2a) The absorption spectra of 3 (0.0275M in DMSO) at different pH values 7.8-12; (2b) compound 9 in DMSO (left) and exposed to A-232 (right) (2c) compound 9 in DMSO (left) and in acidic media DMSO/NaOH (right)

Colour at the initial and final state depends on the initial hydrazone structure and charge delocalisation induced by the deprotonation. The insertion of a strong electron withdrawing group like $-\text{NO}_2$ (4) seems to enhance the colour alteration to blue (576 nm) instead of reddish (497 nm) for reference (1) or purplish (515 nm) for 7 (Table. 1).

Scheme 2. Electronic delocalisation induced by labile hydrogen deprotonation



This visual color alteration seems to be the same for organophosphorus detection. The colour after sodium hydroxide addition in liquid phase was the same for exposition to a Novichok (figure. 2b and 2c). For both Novichok and NaOH, the colour alteration was detected in liquid phase for all hydrazones (Figure 2.). Therefore, UV-visible experiments were conducted with NaOH as it can be assimilated as a simulant for Novichok. Like NaOH, some common interfering compounds like NaOCl, KH_2PO_4 , NH_3 or strong organic base like triethylamine produced a colour alteration in liquid phase. For CWAs, only Novichok agents were detected by hydrazones (Figure 2.) in liquid phase (DMSO).





Spectroscopic data. Changes in absorbance experiments were used to determine dissociation constant values (pKa). From previous UV-visible spectra (Figure 2a.), evolution of the two hydrazone species in equilibrium (Scheme 2.) can be plotted. An example of pKa determination was detailed in supporting information for hydrazone 7 (Figures. S1, S2 & S3). Absorbances at wavelengths before and after colorimetric response in function of pH (Figure S2) show distinctly the evolution of two species. Hydrogenated hydrazone chemosensor (HyH) was predominant from pH 7 to 10.5. From pH 11 to 13.5, two species were in equilibrium. An increase of pH promotes formation of the anionic form of hydrazone chemosensor (Hy^-) at the cost of HyH. In very basic conditions, Hy^- was predominant. This trend was confirmed by the visible colour alteration from yellow to purple-blue (Figure 2c.).

An isobestic point was clearly formed for each hydrazone derivatives, characterised in absorbances vs. pH plotting (Figure S2) by the crossing of the two curves. They are crossing at $\text{pH} = \text{pKa}$. Henderson-Hasselbalch equation (Equation 1.) was used to calculate pKa values. pKa determination was detailed in Supporting Information

$$\text{pKa} = \text{pH} - \log \frac{[\text{Hy}^-]}{[\text{HyH}]} \quad (1)$$

Spectroscopic and pKa data were summarized in the Table 1. pKa values range between 10.9 and 12.7. The lower pKa (10.9) was obtained for *para*- NO_2 substituted hydrazone (3). It can be explained by the presence of the electron withdrawing group $-\text{NO}_2$ at the *para*-position of the phenyl ring. At the opposite, hydrazones containing electron donating group as $-\text{CH}_3$ (2), or $-\text{SCH}_3$ (4) at the *para*-position have higher pKa values, respectively 12.3, 12.6, and 12.6. Additionally, in *meta*-position an electron donating group such as $-\text{OCH}_3$ slightly weakened the pKa value for hydrazone (5) which is a 4-hydroxy-3-methoxyphenyl group. pKa values seem to depend on substituent effects.

Table 1. UV-visible spectral data of Hydrazones (1-11) and acid dissociation constants (pKa)

Entry	Substituents	λ_{\max} protonated form	λ_{\max} deprotonated form	$\Delta\lambda$	pKa
1	R ₁ =R ₂ =R ₃ =H	395	497	102	11,5
2	R ₁ =CH ₃ , R ₂ =R ₃ =H	399	496	97	12,3
3	R ₁ =NO ₂ , R ₂ =R ₃ =H	415	576	161	10,9
4	R ₁ =OSH ₃ , R ₂ =R ₃ =H	411	506	95	12,6
5	R ₁ =OH, R ₂ =OCH ₃ , R ₃ =H	411	506	95	11,8
6	R ₁ =R ₂ =H, R ₃ =CH ₃	392	491	99	11,2
7	R ₁ =NO ₂ , R ₂ =H, R ₃ =CH ₃	404	574	170	11,1
8		389	521	132	11
9		402	549	147	11,4
10		402	531	129	11,6
11		427	523	96	11,8

The trend shows that electro withdrawing groups such as *para*-NO₂ lower pKa values while electro donating groups slightly increase values. Moreover, increase conjugation without adding any additional substituent as for hydrazone chemosensor (7) increase pKa value. Extended conjugation stabilizes hydrazone chemosensor structure and consequently decreases reactivity thus pKa. In addition, steric effects seem to increase pKa value. In comparison, hydrazone (3) which is only *para*-NO₂ substituted has a lower pKa value than hydrazone (7) which is *para*-NO₂ substituted and contained a methyl group at R₃ position, next to the hydrazone bond. The steric effect induced by the methyl group probably prevents the base from approaching the hydrazone bond: the labile hydrogen deprotonation is less favoured, reflected by a higher pKa value.

Hydrazone properties were influenced by their initial structure, added substituents, and also steric effects near the hydrazone bond. As for UV-visible investigations, chemosensor molecular structure directly affects both λ_{\max} and pKa values. *para*-NO₂ substituent seems to promote the most important effect on chemosensor properties. It leads to hydrazone chemosensors with a lower pKa (10.9-11.1) and a stronger colorimetric response ($\Delta\lambda > 160$ nm). The trend was the same for UV-visible values than pKa. Hydrazone containing electron withdrawing group (3 and 7) or strong conjugated one (9, 10 and 11) have a higher $\Delta\lambda$ between their protonated and deprotonated UV-visible values ($\Delta\lambda > 100$). This observation largely depends on hydrazone structure. An electronic depletion, as in hydrazone containing -NO₂, promotes a more conjugated structure. The same statement may be applied for hydrazone derived from carboxaldehyde (9, 10, 11 and).

Any other correlation between UV-visible data and pKa measurements were noticed. In fact, adding a substituent

promotes a bathochromic shift of λ_{\max} of HyH for all hydrazone chemosensors. The trend was not the same for pKa values, it differs from the electro withdrawing or donating character of the carboxaldehyde reactant. An electro donating group such as -CH₃ (2) or -SCH₃ (5) clearly increases pKa value (respectively 12.3 and 12.6) comparatively to reference (11.5). At the opposite for UV-visible data, λ_{\max} of HyH were higher: 499 and 407 nm comparatively to 395 nm for reference (1). However, it can be observed for deprotonated form (Hy⁻) a slightly decrease of λ_{\max} for (2) 496 nm in comparison to reference (497 nm). The difference was too lower to make any correlation with pKa values, all the more this remark was observed only for these two cases.

In terms of Novichok detection, steric effects seem to have a **more** significant incidence than pKa. In sensibility test with Novichok A-232 (Figure S4), 4 hydrazone chemosensors conducted to an earlier and more contrasted color alteration, *para*-NO₂ derivatives (7) and (7) as well as (8) and (10). As already been seen, (3) and (7) lead to contrasted chromogenic response with a good reactivity. A low pKa, respectively 10.85 and 11.2, may be correlated with a larger detected Novichok (A-232) amount. However, as a contrary example chemosensor (11) with a pKa value of 11.4 was the worst chemosensor in terms of sensibility, yet structure chemosensor does not differ from (8). The steric hindrance of the quinoline leads to a drastic diminution of the detected amount of Novichok, in spite of chemosensor has a weak pKa. Setting aside steric effects caused by hindered group, chemosensor (4) with high pKa value: 12.6 (compared to reference (1), pKa= 11.5) detected a low amount of Novichok in comparison with (3).

In other words, to rapidly detected Novichok with a good sensibility and efficacy a chemosensor as (3) may be promising. A more contrasting color response is interesting to avoid confusion and false positive. Hydrazone *para*-NO₂ substituted chemosensor promotes both contrasting color alteration and good sensibility (10 molar equivalents detected). In addition, pKa and steric effects must be considered in Novichok sensing. High pKa value seems to traduce a weaker reactivity and steric hindrance avoid the organophosphorus approach near the reactive centre thus limits the response. These observations will guide future synthesis to obtain more reactive hydrazones.

Detector conception and application. A selection of 6 hydrazones (2, 5, 7, 9, 10, and 11) were used to test the detection device. Liquid CWAs: Sarin, Soman, Tabun, VX, A-230, A-232, A-234, A-242, Sulfur mustard HD, Nitrogen mustard HN₃, Lewisite L₁, Clark 1 and Clark 2 (Chart. 1) were directly added on each coloured spot. 1.2 μ L of toxic was used (400 molar equivalents).

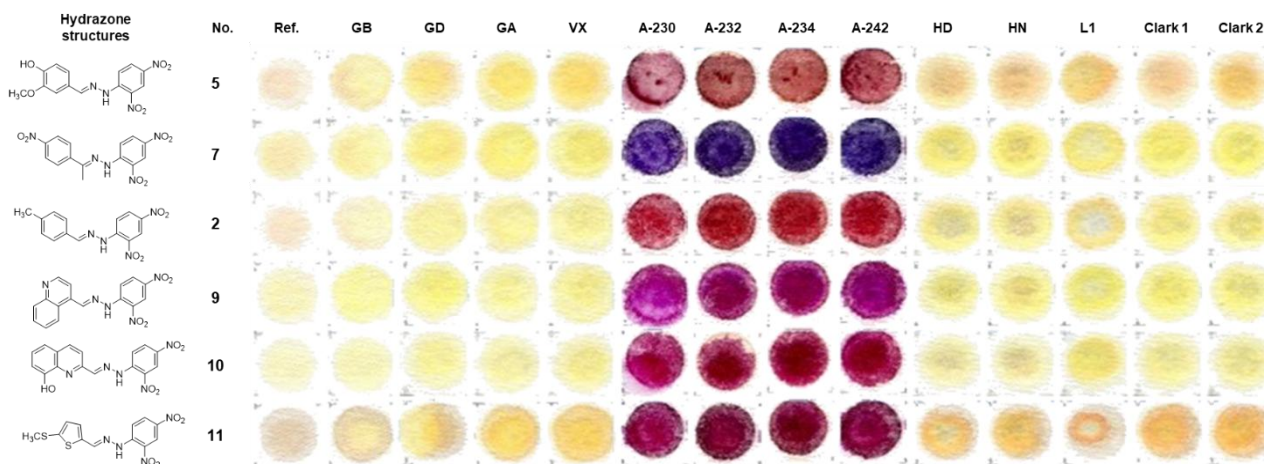


Figure 3: Hydrazone spotted glass fibers papers exposed to 13 various organophosphorus nerve agents; from top to bottom: hydrazone 5, 7, 2, 9, 10, and 11

Spotted hydrazone chemosensors were yellowish before exposure to CWAs. Exposure to the 13 CWAs leads to a noticeable colour change only for the 4 tested Novichok agents A-230, A-232, A-234 and A-242. Hydrazones chemosensors instantly turn from yellow to purplish (Figure 3). No colorimetric response was noticed for the 9 other tested nerve agents (Sarin, Soman, Tabun, Sulfur mustard HD, Nitrogen mustard HN₃, Lewisite L₁, Clark 1 and Clark 2). From experimental data, it can be concluded that the proposed method has a selectivity to the phosphoramido or phosphonamido organophosphorus nerve agents. In other words, there is a total selectivity for Novichok (Figure. 3).

Any tested interfering compounds: water, gasoil, acetic acid, HCl, NaOH, NaOCl, H₂O₂, NH₄OH, KH₂PO₄ and trimethylamine have not induced a colour alteration. These results have been detailed in Supporting Information.

At the opposite, in liquid phase (in DMSO) interfering results were not the same. Potential interferents were tested in the same conditions as for CWAs (0.5 mM in DMSO exposed to a large excess of interferent). Only basic interferents (NaOCl, NaOH, NH₄OH, KH₂PO₄ and trimethylamine as organic base) have induced a colour alteration in liquid phase. An impregnated substrate with a hydrazone solution is a real asset to avoid these interferent issues. This is all the more important as any other CWAs lead to a colorimetric response, even VX in comparison of liquid tests. This colorimetric device allows a very specific detection of phosphoramido and phosphonamido phosphorus agents only.

CONCLUSION

In conclusion, we developed hydrazone chemosensors for the detection of organophosphorous nerve agents. These hydrazones are composed of an electron-deficient 2,4-dinitrophenyl ring π -conjugated by a hydrazone link to an electron-rich aromatic part. Impregnated on glass fibers, chemosensors were highly selective and allowed a

rapid eye-naked detection with a good color contrast. Hydrazone chemosensors were synthesized easily. Detector prototype was developed at low cost and designed without process difficulties. These specific properties promote hydrazone chemosensors as good candidate to complete the detection range of M8 paper or PDF1. Novichok detection will be an asset to quickly react and organize emergency in case of contamination.

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information.** A brief statement in nonsentence format listing the contents of material supplied as Supporting Information should be included, ending with "This material is available free of charge via the Internet at <http://pubs.acs.org>." For instructions on what should be included in the Supporting Information as well as how to prepare this material for publication, refer to the journal's Instructions for Authors.

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Author Contributions

L.T. carried out the experiments and wrote the draft of the manuscript. S.P. and A.C. revised the manuscript. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest

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