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ORIGINAL ARTICLE

DETECTION OF 2-(DIALKYLAMINO)ETHANETHIOLS AND V-SERIES NERVE AGENTS IN WATER AND AQUEOUS SOLUTIONS USING FOLIN-CIOCALTEU REAGENT

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Summary

A simple colorimetric tube detector for the detection of 2-(dialkylamino)ethanethiols and V-series nerve agents in water and in aqueous solutions is proposed. The detector is based on the reaction of the compounds with a chromogenic Folin-Ciocalteu reagent yielding a blue coloured reduction product. The detection limit of 2-(dialkylamino)ethanethiols is 2.5 mg/l, measurement range up to 250 mg/l. The detection limit of the V (VX) agent is 10 mg/l. The method allows V agents and their precursors and hydrolysis products to be easily distinguished from G-series nerve agents.

Key words: colorimetric tube detector, water analysis; V-series nerve agents; 2-(dialkylamino)ethanethiols; Folin-Ciocalteu reagent

INTRODUCTION

Nerve agents (NA), powerful inhibitors of acetylcholinesterase (AChE), are the most important group of lethal chemical warfare agents (CWA). The oldest group of nerve agents is the G-series agents, which include tabun (GA, ethyl-dimethylphosphoramidocyanidate), sarin (GB, isopropyl-methylphosphonofluoridate), soman (GD, pinacolyl methylphosphonofluoridate), cyclosarin (GF, cyclohexyl methylphoshonofluoridate) and other organophosphorus fluoridates. The second important nerve agent group consists of V-series agents, especially the VX agent, i.e. *S*-[2-(diisopropylamino)ethyl]-*O*-ethyl-methylphosphonothioate, and VR agent (R-33), i.e. *S*-[2-(diethylamino)ethyl]-*O*-isobutyl-methylphosphonothioate. Potential nerve agents also include some groups of compounds with a hybrid chemical structure (GV) or so far not very well understood organophosphorus compounds of "Novichok" type [1, 2].

In view of the high toxicity of nerve agents, a highly sensitive biochemical cholinesterase reaction based on the hydrolysis of a suitable substrate (acetylcholine analogues) and indication of the hydrolysis products using chemical or electrochemical methods must be considered as the primary principle of their detection and determination [3]. However, in principle this method does not enable mutual distinguishing

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of organophosphorus nerve agent groups. This is possible only after suitable sample pre-treatment using special procedures based on the different efficiencies of oxime reactivators of AChE [4], or also using other physical, physicochemical and chemical methods with various levels of instrumentation and sensitivity [5-7]. The most frequently used methods include chemical methods based on their nucleophilic substitution with alcoholate or hydroxide ions yielding 2-(dialkylamino)ethanethiols, the determination being usually based on their strong reducing effects accompanied by colour changes. For instance, the compounds reduce iodine, potassium permanganate, 4.4-bis(dimethylamino)benzophenone oximes, quinonoximes or tetrazolium compounds (triphenyl tetrazolium chloride, blue tetrazolium) [8] yielding the corresponding coloured products. Among the best known methods are those based on the reduction of Ellman's reagent or its analogues yielding aromatic thiols of characteristic colour [9, 10]. Some triphenylmethane dyes (guinea green, malachite green), which are discoloured when treated with 2-(dialkylamino)ethanethiols [11], were proposed for the spectrophotometric determination as well. The complex-formation reaction of V agents with Dragendorff's reagent is also well known [8]. Processes based on the formation of ion associates and their extraction and spectrophotometric evaluation (azo dyes, nitrophenols, sulphophthaleins) constitute a specific group of chemical methods [12-14].

It is clear that a modern society puts high requirements on quick and accurate analysis using the optimum procedure at a low cost. The aim of this work was to propose and develop a simple group detection method for 2-(dialkylamino)ethanethiols and V-series nerve agents in water and in aqueous solutions using a tube detector technique, which requires no sample pre-treatment or adjustment of the working conditions. The use of the commercially available Folin-Ciocalteu reagent, which is routinely applied in analytical practice to the detection and determination of phenols and a wide range of other easily oxidisable compounds (amines, alkaloids), was proposed for this detector [15-21].

EXPERIMENTAL PART

Chemicals and Equipment

Folin-Ciocalteu reagent (2 M), sodium carbonate p.a. (both Sigma-Aldrich, USA) and redistilled water were used for the preparation of the packing and detection solutions for the tube detector. Silica gel (Grace, Germany), particle size 0.7-0.9 mm, which had been thoroughly purified with 10 % hydrochloric acid, rinsed with redistilled water to neutral pH, and activated at 130°C, was used as the support. Glass 5 mm i.d. packing tubes, polyethylene sealing and distributing elements, and polyamide gauze (Tejas, CR) were used for setting up the detector. 98.3% 2-(diisopropylamino)ethanethiol hydrochloride (DIAET, University of Defence, CR), 95% 2-(diethylamino)ethanethiol hydrochloride (DEAET), and 95% 2-(dimethylamino)ethanethiol hydrochloride (DMAET), both Sigma-Aldrich, USA, were used as model compounds. *S*-[2-(diisopropylamino)ethyl]-*O*-ethyl-methylphosphonothioate, i.e. VX agent (University of Defence, CR) served as a real sample. Spectrophotometric and colorimetric measurements were carried out on an Aquamate instrument (Thermo Electron, UK) and on a LMG 173 portable tristimulus colorimeter (Dr. Lange, Germany).

Working Procedures

Detector Preparation

The detector consisted of a glass 5 mm i.d. packing tube filled with an indication layer 15 mm long and a vial with the detection solution. The indication layer was prepared by impregnating silica gel with a 20% aqueous solution of Na_2CO_3. The consumption was 110 ml of the solution per 100 g of silica gel. The impregnated mixture was dried at 110°C for 4 hours. The indication layer was fixed in the detector by means of polyethylene elements and polyamide gauze. The vial filled with Folin-Ciocalteu reagent was placed in the detector above the indication layer and both ends of the detector were sealed.

Detector Testing and Evaluation

Stock solutions prepared by dissolving 0.1 g of the respective 2-(dialkylamino)ethanethiol (DMAET, DEAET, DIAET) in 100 ml of water were used to test the detector. Working solutions were prepared by dilution. Freshly prepared solutions were always used for the experiments.

The detector was opened by breaking off both tips and immersed into the liquid sample. When the detection layer was saturated with the sample, the vial was crushed and its content was lightly shaken down onto the indication layer. The colour change in the indication layer was evaluated visually in daylight. This method was applied to the calibration of the detector and preparation of the standard (colour intensity dependence on concentration), to the study of the dependence of the detector response on the concentrations of the reagent and of Na_2CO_3, as well as to the study of the time dependence of the colour intensity (using the tristimulus colorimeter with the fixed detector) and of interfering effects.

The limit of detection was established as the lowest concentration of 2-(dialkylamino)ethanethiols at which the detector colour change could be perceived visually. The limit of detection was identified by measuring a series of 20 samples of a given concentration for the probability level P = 0.05 (95% confirmation of concentration). The upper limit of the measuring range was identified with the maximum concentration of 2-(dialkyl-amino)ethanethiols which could still be visually proportionally distinguished. The method precision was tested by repeated measurement of 5 replicate samples during one day (intra-day precision) and on 3 different days (inter-day precision). The accuracy of the method was verified by measuring a series of samples with different concentrations and comparing the results with the standard.

$$\mathbf{Na_3[PMo_{12}O_{40}] + 4\ NaSCH_2CH_2NR_2 \rightarrow Na_7[PMo_4Mo_8O_{40}] + 2\ S_2[(CH_2CH_2NR_2)_2]}$$

Figure 1. Scheme of the reaction of Folin-Ciocalteu reagent with 2-(dialkylamino)ethanethiols

RESULTS AND DISCUSSION

Reaction Principle and Colour Characteristics

The colorimetric tube detector of 2-(dialkylamino)ethanethiols and V-series nerve agents is based on their reaction with Folin-Ciocalteu reagent yielding a blue coloured complex. During this reduction reaction, aminothiols are oxidised presumably to the corresponding disuphides. A general reaction scheme is shown in Figure 1. An absorption spectrum typical for DMAET, DEAET and DIAET, with the absorbance of maximum 650 nm, is shown in Figure 2.

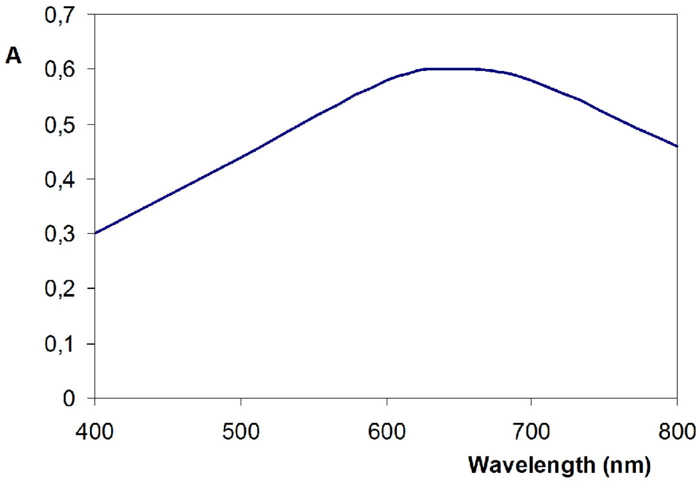

Figure 2. Typical absorption spectrum for the product of the reaction of Folin-Ciocalteu reagent with 2-(dialkylamino)ethanethiol

Colour Stability

Colour developed on the indication layer virtually instantly after crushing the vial with the reagent. The maximum colour intensity, verified using the tristimulus colorimetry method, was observed in approx. 8 minutes (Figure 3). The colour was stable for 24 hours as a minimum.

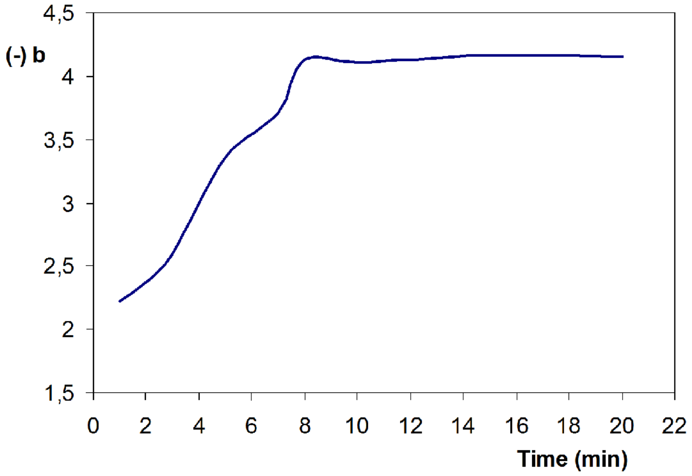

Figure 3. Colour intensity dependence on time, concentration of DIAET 50 mg/l, tristimulus colorimetry method; *(-)b* is a negative value of the parameter *b* (Lab colour system)

Reaction Conditions

The effect of different experimental conditions on the detection result was studied. Folin-Ciocalteu reagent is known to produce colour with the reducing agent only after adding a suitable alkali (usually sodium hydroxide, sodium carbonate). This essential condition was met in the tube detector, as it contained the indication layer impregnated with sodium carbonate and the vial with Folin-Ciocalteu reagent solution. First the indication layer was saturated with the sample and then the liquid reagent was added by crushing the vial.

As shown in Table 1, the minimum sodium carbonate concentration in the indication layer needed for a correct course of the detection process was 10%. A lower concentration decreased the intensity of the blue colour or the colour was not observed at all. The optimum sodium carbonate content was 20%, which also provided a high resistance of the detector to acidic compounds in the sample.

As expected, the optimum results and good stability in the vial were achieved with the undiluted commercial Folin-Ciocalteu reagent. Dilution of the reagent resulted in a decrease in the colour intensity of the reaction product.

All basic experiments were carried out at a sample temperature of 18-25°C. Temperature increase (up to 50°C) was found to lead to an increase in the reaction product colour intensity, in line with published data [18].

Detection Data

The intensity of the blue colour in the indication layer was clearly related to the concentration of 2-dialkylaminoethanethiols in the water. The colouring is shown in Table 2. The colour standard

Table 1. Colour in dependence on the sodium carbonate concentration (2-(dialkylamino)ethanethiol concentration 50 mg/l, measured after 10 minutes)

Sodium carbonate concentration, %	Indication layer colour
1	Yellow to greyish
5	Yellow to light blue
10	Blue
15	Blue
20	Blue

Table 2. Dependence of detector colouring on 2-(dialkylamino)ethanethiol concentration

Concentration, mg/l	Colouring
0	Yellow to greyish
2,5	Bluish
5	Light blue +
10	Light blue ++
25	Light blue +++
50	Blue
100	Intensive blue
250	Very intensive blue
500	Very intensive blue

was prepared using the PANTONE system [22]. The limit of detection was 2.5 mg/l for all the 2-(dialkylamino)ethanethiols which were studied. The upper limit of the visual proportional determination range was 250 mg/l. When testing the method precision and accuracy, virtually no visually distinguishable differences were observed between the results.

Detection of VX Agent in Water

The method was applied to the detection of nerve agents. A fresh solution of VX agent in distilled water was used as the model for the study. The lowest concentration of VX agent in water detected by the device was 10 mg/l. This colouring of the indi-cation layer by VX agent corresponds to the colouring effect of DIAET at 2.5 mg/l (Table 3). This means that the detector is able to determine the upper limit of permissible concentrations of V agents in water according to Allied Standard STANAG 2136 [ref. 23]. This standard says that if water is contaminated with V agents at a concentration exceeding 10 mg/l, such water cannot be used even after treatment. The blue colour of the indication layer is likely to be produced not only by the hydrolysis product (DIAET) but also by the VX agent itself.

Table 3. Detector colour at various VX agent concentrations in water

VX agent concentration, mg/l	Colour	Note
100	Deep blue	Colour corresponds to 25 mg/l of DIAET
20	Light blue	Colour corresponds to 5 mg/l of DIAET
10	Bluish	Colour corresponds to 2.5 mg/l of DIAET

Interfering Effects

Interactions of Folin-Ciocalteu reagent with other chemical warfare agents and military chemicals were also investigated within this study. The assumption that any compound containing aminic nitrogen or arsenic would yield a blue reaction product was not confirmed. Among the whole range of chemicals studied, positive results were only obtained with the irritants adamsite and capsaicin. An overview of the studied compounds and their tolerance limits is included in Table 4.

Detector Service Life

The performance of the tube detector was tested periodically over 6 months by using samples of 2-(dialkylamino)ethanethiols in water. If kept at temperatures up to 30°C and protected from direct sunlight, the indication properties of the detectors did not change during this period. In view of the verified stability of the components and of the indication packing with sodium carbonate, and with Folin-Ciocalteu reagent in the vial, the actual service life of the detector can be estimated at several years.

CONCLUSION

The simple colorimetric detector with Folin-Ciocalteu reagent for the detection of 2-(dialkyl-amino)ethanethiols and V-series nerve agents in water and in aqueous solutions is suitable for field use in chemical surveys and chemical control or for the implementation of the Chemical Weapons Convention. The detector showed a characteristic blue colour in the presence of the above compounds. Although only at a guidance (semi-quantitative) nature, the method provided reproducible and reliable results. No sample pre-treatment was required. The device was able to detect 2-(dialkyl-amino)ethanethiols at a minimum concentration of 2.5 mg/l and VX agent (non-decomposed) at a minimum concentration of 10 mg/l. In case of a positive enzymatic test, the detector allows V agents to be distinguished from G agents (sarin, soman, tabun, cyclosarin). Among the examined chemical warfare agents and military chemicals, adamsite and capsaicin were the only appreciable interferents.

Table 4. Tolerance limits for some military chemicals in DIAET (10 mg/l) detection; the tolerance limit is the highest concentration of a foreign compound that can be present in the sample without interfering with the detection

Agent	Tolerance limit, mg/l
Capsaicin	>10
Adamsite	>20
BZ-agent	>50
Nitrogen mustard (HN-3)	>50
Pelargonic acid morpholide	>80
Lewisite	>100
Sulphur mustard (HD)	>150
Diphosgene	>200

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