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In situ determination of nerve agents in various matrices by portable capillary electropherograph with contactless conductivity detection

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ABSTRACT

Rapid, efficient and robust methods for sampling and extracting genuine nerve agents sarin, soman and VX were developed for analyzing these compounds on various solid matrices, such as concrete, tile, soil and vegetation. A portable capillary electrophoretic (CE) system with contactless conductometric detection was used for the *in situ* analysis of the extracted samples. A 7.5 mM MES/HIS-based separation electrolyte accomplished the analysis of target analytes in less than 5 min. The overall duration of the process including instrument start-up, sample extraction and analysis was less than 10 min, which is the fastest screening of nerve agents achieved with liquid phase separation methods to date. The procedure can easily be performed by a person in a protective suit and is therefore suitable for real-life applications. The CE results were validated by an independent GC–MS method and a satisfactory correlation was obtained. The use of a proper sampling strategy with two internal standards and "smart" data-processing software can overcome the low reproducibility of CE. This has a significant impact on the potential acceptance of portable CE instrumentation for the detection and analysis of genuine chemical warfare agents (CWA).

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1. Introduction

Chemical warfare agents (CWA) are chemical substances that are intended for use in military operations to kill, seriously injure or incapacitate people through their physiological effects. As a result of ongoing efforts to outlaw the production, stockpiling and use of chemical weapons, a Chemical Weapons Convention (CWC) [1] was enacted in 1997 and has been signed by 188 countries to date. According to the CWC definition, CWA are Schedule 1 substances, i.e. they can be used either as chemical weapons or in the manufacture of chemical weapons; they have very limited or no use outside of chemical warfare. A significant group of Schedule 1 substances are the so-called nerve agents (NA), which can be subdivided into two main classes – G-type and V-type. Nerve agents typically act as efficient acetylcholinesterase inhibitors and attack the human nervous system, resulting in eventual death if an appropriate antidote is not administered in time. Nerve agents were used during World War I, but also more recently in documented cases such as in the Kurdish village of Birjinni (1988) [2], the Matsumoto city incident (1994) [3] and the Tokyo subway attack (1995) [4]. The latter incidents led to several deaths and affected thousands of people.

These types of civil terrorism are probably the reason that nerve agents are being intensely studied and that the search for possible new methods of determining NA is still very active [5]. The quest for the development of an analytical method is targeted at the ability to identify the agents used in an attack as quickly as possible, so that appropriate antidotes can be administered in time. For such a method to be useful, several factors have to be considered, including the portability of the instrumentation, the instrument start-up time, the sampling and sample preparation time, and the actual analysis time.

A large number of analytical techniques have been developed to detect CWA or their degradation products. Simple colorimetric tests are available commercially for the protection of military personnel [6]. At the moment, however, the most popular instrumental technology seems to be ion mobility spectroscopy (IMS). It is an efficient tool for monitoring gaseous phase CWA and provides an effective and rapid method of detection [7]. Other interesting technologies are flame photometry (FPD), and surface acoustic wave (SAW) and surface plasmon resonance (SPR) [6]. Research and development in the field of CWA determination has produced many commercial products; portable instruments are available from large international corporations such as Dräeger, Smiths Detection, RAE Systems and Proengin. Most of these instruments use IMS, FPD or SAW technology platforms. The advantages and disadvantages of many off-the-shelf instruments were documented in a recent report [8]. One common drawback of gas phase detectors is that they are not very effective for detecting aqueous phase CWA. The agent is often deposited on a surface close to the attack site and is subject to hydrolysis due to atmospheric or soil humidity. The

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effectiveness of gas phase detectors is questionable under these circumstances.

Chromatographic separation techniques play a dominant role in many areas including NA detection. Usually, GC [9] and HPLC [10] are coupled to an element specific detector (FPD) [11–13] or to a mass spectrometer (MS) [14–16] because these detection techniques provide very reliable identification of separated compounds. Each of these techniques has advantages and drawbacks for on-site analysis. For instance, GC can be made portable and the separation times are relatively short [17]. However, the analysis typically requires derivatization steps because NA are not sufficiently volatile, and analysis from an aqueous environment presents a particular challenge. HPLC, on the other hand, can be used for the analysis of aqueous extracts, but it cannot readily be made portable and thus requires the sample to be delivered to an analytical laboratory. It is therefore not suitable for rapid on-site screening.

Electromigration methods such as capillary electrophoresis (CE) have attracted only academic interest as possible technological platforms for NA detection. No off-the-shelf instruments have yet appeared on the market, probably due to the perceived immaturity of the technique. However, CE has some distinct advantages, which have been demonstrated in many publications. It is arguably the technique that is best suited for field and on-site analysis of complex mixtures in complicated matrices. CE can easily be miniaturized, unlike GC or HPLC. CE, especially with electrochemical or optical (LED based) detection, does not consume large quantities of energy. CE startup time is significantly shorter than that of HPLC, because there is no requirement for lengthy equilibration of the separation column with an eluent. In addition, CE analysis times are unquestionably the shortest of the available separation techniques.

It is therefore not surprising that CE is very popular for the analysis and screening of nerve agents and their degradation products, as documented in several recent reviews [18–21]. The prevailing CE detection methods are UV and conductivity detection, but laser-induced fluorescence [22–25], MS [26–29] and element specific detectors [30,31] have also been used. CE separation with indirect UV detection of NA degradation products was first described by Pianetti et al. in 1993 [32]. Phenylphosphonic acid was used as the UV-absorbing probe, as most NA degradation products do not absorb in UV, and this technique was subsequently adopted by other authors [33–35]. Alternative electrolyte probes applied in indirect UV detection include sorbate [36,37], borate [38,39] and chromate [40]. Derivatization with sodium borate and direct UV detection at 214 or 254 nm was advanced by Robins and Wright [39]. All of the reported research on CE with UV detection relies on laboratory-based instruments with UV detectors that consume large quantities of energy. Although CE systems with diode-based UV detectors can easily be made portable, they have not yet been used for CWA detection.

Conductivity detection is probably best suited to an on-site, portable CE device, because it can be miniaturized, power consumption is minimal, and it is relatively sensitive to the compounds of interest, especially NA degradation products. Contact conductivity detection with CE for the detection of NA degradation products was first demonstrated by Rosso and Bossle [41] and later by Nassar et al. [33,34]. Due to the obvious advantages of the recently developed contactless conductivity detection (C4D) approach [42,43], contact conductivity detection has been replaced by C4D in CE. A separation electrolyte based on MES/HIS has been used in CE with C4D for NA separation. A negative [44–47] or positive [48–51] polarity mode was applied; polarity in this context refers to the polarity on the detection side. Some researchers added an electroosmotic flow modifier to the electrolyte to suppress or reverse the EOF [33,34,49–51], while others used no EOF modification [44–47]. The negative polarity mode offers some advantages over the positive, as the inorganic cations and anions are effectively separated from the NA degradation products. In the positive polarity mode, the small anions, if present in the samples in significant amounts, may influence the separation of the analytes of interest, and pretreatment steps are often needed to remove the small anions and the cations from these samples [40].

Of the approximately 40 publications in which the use of capillary electrophoresis for the detection of CWA is reported, only three applied it for the determination of genuine nerve agents [25,33,34] rather than their simulants or degradation products. The analyses were conducted in the laboratory, not in the field. This is understandable, because nerve agents are not freely available, even for research purposes. There are severe restrictions on their use (a license from state authorities is often needed), and NA require special attention during subsequent disposal/decontamination. Only a few sites in Europe, for example, are authorized to perform such research and have suitable disposal facilities. Research conducted with NA degradation products or simulants certainly has great importance, but the performance of CE with genuine nerve agents under real life conditions is largely unknown. This is especially relevant to information on NA sampling and extraction methods for CE analysis from different matrices in which NA might possibly be used.

To the knowledge of the authors, there are no reported cases of the use of CWA detectors in the battlefield or immediately following a terrorist attack. On the contrary, there are documented cases of samples from suspected sites such as the Tokyo subway attack and the other incidents described above being analyzed *post factum*. The dissemination of CWA is highly dependent on atmospheric conditions and it is difficult to achieve effective dispersion. The most probable analytical scenario will require analysis of an agent which is deposited on a surface close to the site of application, and which will degrade rapidly due to atmospheric or soil humidity. The effectiveness of gas phase detectors is questionable under these circumstances. Liquid samples from the suspected site can be transported to the laboratory for GC–MS or HPLC–MS analysis and identification. However, this causes a significant time delay that may be critical in cases that require a rapid response. *In situ* analysis with robust analysis protocols and minimum sample preparation will be highly desirable. The “Guide for the Selection of Chemical Detection Equipment for Emergency First Responders” [6] lists about two hundred instruments for the detection of chemical weapons, most of them handheld or hand portable, which are designed to be used in field. Surprisingly, except for semi-qualitative colorimetric kits, only a few are suitable for liquid or solid samples (such as FPD instruments which scrape and heat the collected soil or Raman spectroscopy-based instruments). Portable field instruments based on CE technology could provide a viable solution in these situations.

In this paper, four main objectives were addressed that could have a significant impact on the acceptance of portable CE instrumentation for the detection and analysis of genuine NA. First, we conducted experiments to support the results obtained from CE analysis of NA degradation products, by sampling and identifying the genuine nerve agents sarin, soman and VX in a real-life situation using a portable CE instrument with C4D. Second, we developed robust, rapid and efficient extraction procedures that could be used by an individual wearing a protective suit. Third, we sought to validate the CE findings with results obtained from GC and HPLC. To the best of our knowledge, no such research has been previously reported in the published literature. Fourth, by using an effective sampling strategy with two internal standards and “smart” data-processing software, the low reproducibility that is frequently considered to be the main disadvantage of CE can be overcome.

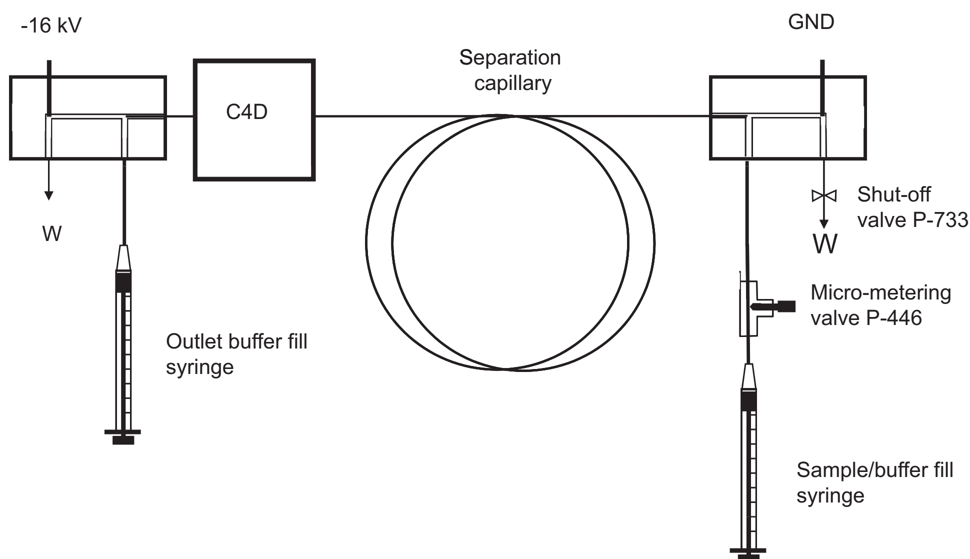

Fig. 1. Schematic of a portable CE instrument with C4D.

2. Experimental

2.1. Materials and methods

2.1.1. Electrophoretic system

A purpose-built portable CE instrument was fitted into a watertight, crush-proof, dust-proof case made of durable plastic (Peli 1200 Case©, Peli Products, Barcelona, Spain). The instrument size was 30 cm × 30 cm × 15 cm and its weight was approximately 5 kg. The instrument was equipped with an HV safety interlock and included a negative high-voltage power supply (EMCO, Sutter Creek, CA, USA) capable of delivering voltages up to −25 kV, an in-house built C4D detector operating at 200 kHz and a voltage of 60 V_{p-p}, and a in-house built data acquisition system based on LTC2440 high speed differential delta-sigma converter (sampling rate 10 Hz, resolution 24-bit). The instrument is controlled and the signal obtained through a USB connection with in-house written, software using a Netbook computer. The instrument is powered by 10 rechargeable AA-batteries, which provide more than 4 h of operation time. A single electrophoretic run takes approximately 5 min; the number of samples that can be analyzed with the stated power capacity is approximately 50. This is sufficient for the analysis of several NA samples on various matrices, as well as the identification and quantification of the analytes. There is no built-in thermostatic control; however, the instrument case is closed during the analysis, which provides sufficient thermal insulation for a single electrophoretic run. Minor EOF fluctuations are corrected by means of two internal standards. In addition, recently developed software for data normalization [52] was applied for peak identification, baseline drift correction and the improvement of injection precision [53].

Fused-silica (FS) capillaries (75 μm I.D., 375 μm O.D., 35 cm total length, 27 cm effective length, Polymicro Technologies Inc. AZ, USA) were used for the separation. Prior to the first use, the separation capillaries were preconditioned with 0.1 M NaOH for 30 min, deionized water for 10 min, and background electrolyte (BGE) solution for 10 min. Before each analysis sequence, the capillaries were manually washed with approximately 100 column volumes of 0.1 M NaOH, 150 column volumes of DI water and 150 column volumes of the background electrolyte, using a syringe. Each of these steps took approximately 0.5–1 min. The rinsing step was followed by high voltage conditioning of the capillary for 2 min. The total time to prepare the instrument before the first analysis was approximately 5 min. Between two successive injections, the capillary was flushed with 100 column volumes of the BGE solution (1 min). At the end of each day, the capillaries were washed with at least 150 column volumes of DI water and kept in DI water overnight. This procedure allowed for a quick start-up, as described above.

2.1.2. Injection

The experimental set up is shown in Fig. 1. The sample injection apparatus includes a splitter interface machined to 35 mm × 15 mm × 15 mm from a block of polyimide. The splitter interface has a 2-cm-long horizontal flow-through channel of 1 mm I.D. to which two vertical channels of the same diameter are connected. A separation capillary is inserted into the side of the horizontal channel and its tip is positioned exactly at the intersection with the first vertical channel. A grounding Pt electrode is inserted into the second vertical channel. Both the capillary and the Pt electrode are secured with 1/16″ flangeless fittings (Upchurch). The injection syringe is connected to the inlet (the first vertical channel in the interface) via a micro-metering valve (P-446, Upchurch Scientific Oak Harbor, WA, USA). The function of the micro-metering valve is to restrict the manually applicable pressure and thus achieve uniform sample flow rates through the splitter interface. Its function and details on the operation are described in a previous publication [53]. During the injection process, a 500 μL volume of sample is first injected manually into the splitter interface with a 1 mL disposable plastic syringe (Omnifix 100 Duo, Braun, Melsungen, Germany), followed by an injection of 500 μL of the background electrolyte solution to remove any remaining sample from the splitter interface. Only an infinitesimal part of the 500 μL sample and buffer volume is hydrodynamically introduced into the separation capillary, while the majority of the volume is directed to the waste.

The outlet side of the second vertical channel is connected with 10-cm-long, 700 μm I.D. PTFE tubing to a waste reservoir. A shut-off valve (Upchurch, P-733) connected to the waste line of the first splitter interface is used for pressurized capillary flushing using the inlet syringe. The other end of the separation capillary is inserted into the second, identical, interface with the high voltage Pt electrode and a wash syringe is connected to the vertical channels of the interface. The outlet side of the second vertical channel is connected to the second waste reservoir.

2.1.3. GC–MS and LC–MS

An Agilent 6890N GC chromatograph with an Agilent 5975B MS detector was used for comparative data analysis. The column was a 30 m × 0.25 mm × 0.25 μm HP-5MS, with He as the flow gas at a constant velocity of 0.9 mL/min. An Agilent 1200 Series LC chromatograph with a 6410 Triple Quadrupole MS detector was used for HPLC analysis. Agilent, Zorbax Eclipse XDB, 150 × 4.6 mm, 5 μm particle size, with aqueous eluent containing trifluoroacetic acid (TFA) was used at a flow rate of 0.4 mL/min.

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and deionized (DI) water was used throughout. Stock solutions of NA degradation products (10 mM) were prepared from acids (butylphosphonic acid, BPA, Alfa Aesar; propylphosphonic acid, PPA, Alfa Aesar; methylphosphonic acid, MPA, Sigma–Aldrich) or their sodium salts (pinacolyl methylphosphonate, PMPA; ethyl methylphosphonate, EMPA; 2-aminoethyldihydrogenphosphonate, AEDHPA, all from Sigma–Aldrich). Isopropylmethyl-phosphonic acid (IMPA) was purchased as a 1000 mg/L methanolic solution (Cerilliant Corp., TX, USA). Salicylic acid was of p.a. quality and purchased from Sigma–Aldrich. All multi-ion standard solutions were freshly prepared from these stock solutions and diluted with DI water. Background electrolyte (BGE) solutions for CE measurements were prepared daily from 100 mM stock solutions of 2-(N-Morpholino)ethanesulfonic acid (MES, Sigma–Aldrich) and L-histidine (HIS, Sigma–Aldrich). Sarin (purity >99%), soman (purity >96.7%) and VX (purity >90.3%) was supplied by the staff of the testing site near Vyškov (Czech Republic) under the licence of the Ministry of Defence and Armed Forces of the Czech Republic.

2.3. Sampling and extraction procedures

2.3.1. Application and sampling of nerve agents

The extraction procedures were developed and the robustness of the sampling procedures was validated at a testing site near Tallinn, Estonia using NA degradation products and the help of staff of the Pioneer Battalion of the Estonian Army. Field experiments with genuine NA were performed in September 2010 at a testing site near Vyškov (Czech Republic). The weather conditions at the testing site were as follows: low pressure and cloudy, with a temperature of about 9 °C. NA application and sample extraction were performed by trained personnel at the test site, and the actual CE analysis of the NA was done by the authors in the vicinity of the sampling site. Five different matrices (Teflon, ceramic tile, concrete, grass and soil) with an equal surface area of 25 cm^2 were contaminated with 100 mg of pure nerve agent (sarin, soman, VX) and kept outdoors (temperature 9 °C), one batch for 30 min and the other for 3 h. After the elapsed exposure time, two procedures were used as described below.

2.3.2. Extraction procedures

Teflon, ceramic tile, concrete matrices: After application of the nerve agent, the surface of the matrix was carefully wiped with a DI water pre-moistened Ghost wipe tissue (Environmental Express, Mt. Pleasant, SC, USA) using tweezers. The tissue was placed into a plastic sample vial containing 10 mL of DI water. The NA hydrolysis products as well as the unhydrolyzed NA were extracted by vigorous shaking for 1 min. The extract was filtered through a 0.45 μm filter (Filtropur S, Sarstedt, Numbrecht, Germany). Internal standards (400 μM AEDHPA, 100 μM salicylic acid) were added to the filtered sample, which was then directly injected into the CE system.

Soil and grass matrices: The matrices consisted of 20 g of sandy soil and 1 g of finely cut grass, respectively. After application of the nerve agent, the samples were placed into a plastic sample vial with 10 mL of DI water and extracted by vigorous shaking for 1 min, followed by filtration through a 0.45 μm filter, the addition of internal standards and direct injection into the CE system.

The total extraction time for each matrix was no longer than 5 min.

3. Results and discussion

3.1. Aqueous extraction of nerve agents

It is well known that nerve agents undergo rapid hydrolysis in aqueous solutions; this is typically a two-step process, as shown in Fig. 2. In the first step, the O-alkyl-phosphonofluoridates (sarin, soman) quickly hydrolyze to isopropyl methylphosphonate (IMPA, the hydrolysis product of sarin) or pinacolyl methylphosphonate (PMPA, the hydrolysis product of soman). O-Alkyl-S-(2-dialkyl-aminoethyl)alkylphosphonothiolates (VX) follow different hydrolysis patterns depending on the pH of the solution. At pH <6.5, as in the case of extraction with deionized water, the predominant reaction is the P-S cleavage [54] that results in the formation of ethyl methylphosphonate (EMPA) and diisopropylaminoethanethiol (DIAET). In the second hydrolysis step, which is typically much slower than the first, all three alkylphosphonates further degrade to methylphosphonate (MPA) and alcohol.

As both sarin and soman are fairly soluble in water, it was expected that aqueous extraction would yield high recoveries of the degradation products for most of the matrices. The solubility of VX in water is rather low; however, below 9.4 °C, VX becomes fairly soluble as well [55]. As the average outside temperature at the sampling site did not exceed 9 °C, high recoveries for VX were also expected. In all matrices, the respective alkylphosphonates from the first hydrolysis step were found in sufficient quantities; however, no MPA peak appeared even in the samples that were exposed to NA for 3 h. These results are indicative of the speed of hydrolysis, as discussed above. The type of nerve agent can thus be identified based on the presence of the hydrolysis product from the first hydrolysis step.

3.2. Separation of the hydrolysis products of nerve agents

The background electrolyte used for the separation of the NA degradation products was comprised of an equimolar mixture of MES and HIS. Separation of all selected alkylphosphonates including internal standards was achieved in less than 5 min in a 7.5 mM MES/HIS electrolyte with a pH of 6 at −16 kV. The separation of the analytes in this research was counter-electroosmotic, e.g. the negative electrode was placed on the detection side. Because phosphonic acids are deprotonated and migrate as anions against the EOF, the slowest migrating analyte, PMPA, will appear first in the electropherogram, while the fastest one, MPA, will appear last. The measured peak areas will also increase accordingly, as the conductivity of the separated acids increases with increasing mobility. The counter-electroosmotic separation mode with negative polarity (on the detection side) has some advantages over the positive polarity mode used by other authors [48–51]. First, there is no need to add EOF modifiers, such as CTAB, into the background electrolyte. Second, there is no interference from small inorganic anions, which are often present in aqueous samples and extracts from environmental matrices. In the negative polarity mode small anions do not reach the detector, because they migrate in the opposite direction to the EOF, at velocities exceeding it. The separation of standard solutions of PMPA, IMPA, EMPA, MPA and two internal standards

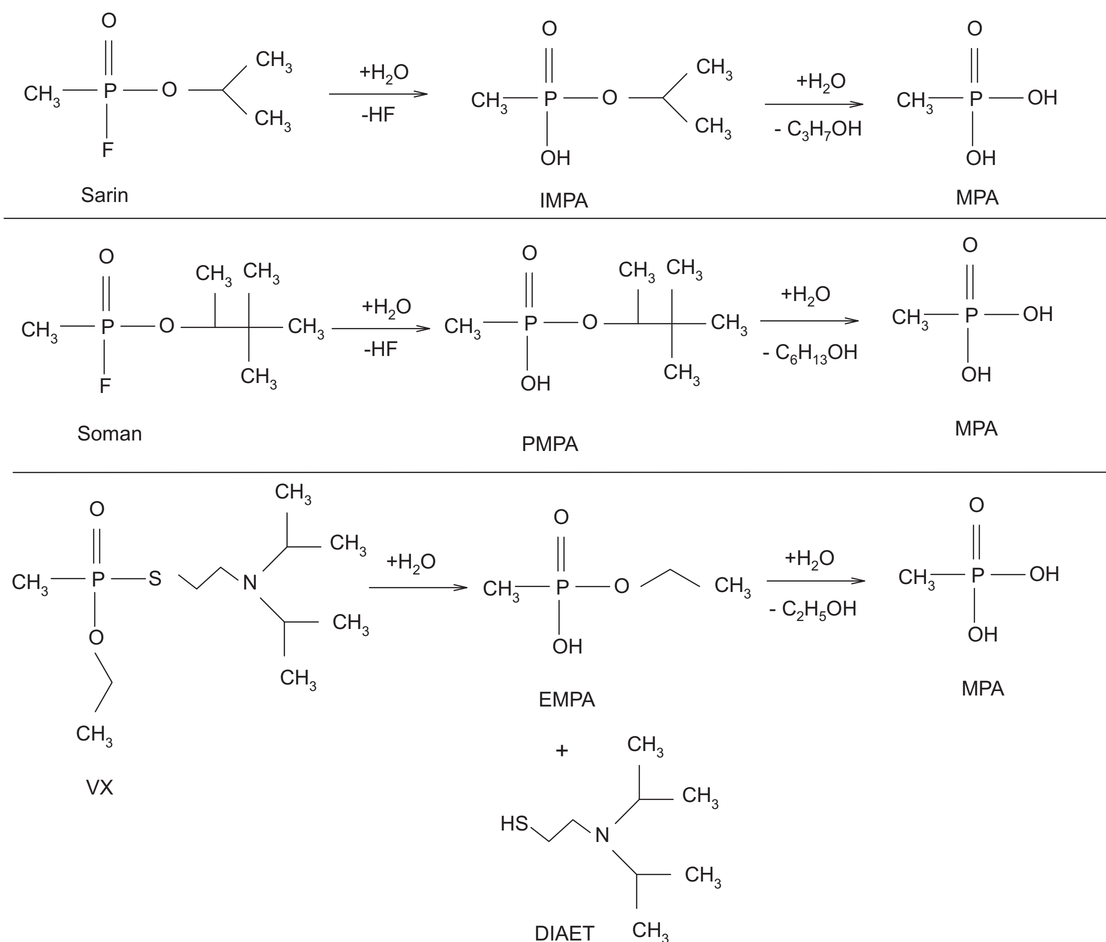

Fig. 2. Aqueous hydrolysis paths of nerve agents sarin, soman and VX.

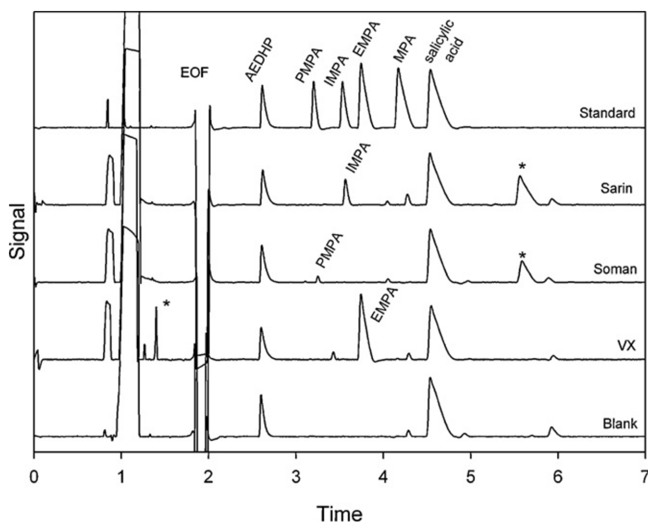

Fig. 3. Separation of hydrolysis products of nerve agents sarin, soman and VX in a concrete matrix. CE conditions: Separation voltage: −16 kV, Background electrolyte: 7.5 mM MES/HIS, pH 6, C4D detection. Injection: 500 μL of standard solution followed by 500 μL of BGE.

(AEDHPA and salicylic acid) is shown in Fig. 3. Two internal standards were selected so that they would effectively bracket the analytes to facilitate their qualitative identification. A previously developed signal processing algorithm [52] was used for baseline correction, signal alignment, peak identification and integration. Unwanted shifts in the time axis caused by variations in electroosmotic flow were corrected based on the premises advanced by Reijenga et al. [56] By linking peaks of two internal standards, these variations can be overcome, and corrected electropherograms were obtained, free from any migration time bias due to minor fluctuations of EOF.

Fig. 3 shows the separation of a standard solution, extracts of sarin, soman and VX obtained from wipe-sampling a concrete matrix, and also a blank solution of the same matrix to which no nerve agent was applied. The figure shows an excellent match between the transformed migration times of the standards and the nerve agent hydrolysis products from aqueous extracts. Note that the electropherograms were recorded on three consecutive days, demonstrating excellent reproducibility of the portable CE system and the effectiveness of the software data processing.

3.3. Calibration linearity, limits of detection

The calibration solutions for quantitative determination of the NA hydrolysis products were prepared in the range of 25–150 μM. AEDHPA at a concentration of 400 μM and salicylic acid at a concentration of 100 μM were added to each standard solution. The calibration curves for raw peak areas were linear throughout the measured range, with coefficients of variation better than 0.94. The linearity was significantly improved by applying a correction with the first internal standard (AEDHPA). By dividing the respective analyte peak areas by the peak area of the AEDHPA, the coefficients of variation improved and were better than 0.98. The second internal standard, salicylic acid, was used for purposes of identification

Table 1
Calibration data for the determination of NA degradation products.

Degradation product	RSD, %	$(a \pm s_a)$[a]	$(b \pm s_b)$[a]	R^2	LOD[b], μM
PMPA	6.2	9.20 ± 0.23	−80.0 ± 2.7	0.9853	25.8
IMPA	6.8	10.02 ± 0.28	−104.2 ± 24.2	0.9815	26.1
EMPA	5.1	18.07 ± 0.38	−117.1 ± 32.7	0.9895	15.2
MPA	8.3	17.24 ± 0.36	−118.6 ± 30.7	0.9898	16.0

[a] Calibration line equation $y = a + bc$ parameters: a – intercept, s_a its standard deviation, b – slope, s_b its standard deviation; y – detector response c – concentration.
[b] Limit of detection calculated as three times the standard deviation of the noise level.

but not for quantitation, because in some samples, a co-migration of unidentified matrix peaks with salicylic acid was observed. The correction procedure was legitimate, because one internal standard is usually sufficient to achieve reasonable precision of the measured data [57] with CE hydrodynamic injection. The calibration data including calibration ranges, linearity and limits of detection are shown in Table 1.

3.4. Analysis of different matrices

Different matrices were analyzed as described in the section on details of the experiments. They included one reference matrix (Teflon) on which the absorption of NA was expected to be negligible. The other matrices included concrete and ceramic tile, which represent typical solid surfaces that are found in locations where there is a greater probability of NA usage, such as urban and public areas with high population concentrations. On the other hand, soil and vegetation represent types of samples that would be found in rural areas, and in which higher adsorption of NA would be expected. The latter matrices cannot be sampled by simple surface wiping techniques, as the NA penetrate deep into the matrix. Aqueous extraction was selected for these samples. Duplicate experiments with different exposure times (30 min and 3 h after contamination, respectively) were performed on each matrix. Fig. 4 shows a separation of an aqueous extract of sarin (A) and VX (B) from all matrices. For each matrix, a typical hydrolysis product peak appears that can be used to identify the nerve agent. The IMPA peak for sarin was observed in all extracts from all matrices and was quantified using the previously described calibration procedure with one internal standard. Soman produced a similar eletropherogram with a PMPA peak appearing for all matrices (data not shown). A large, unknown peak (marked with an asterisk) occurred for all matrices contaminated with sarin and soman. This peak is not fluoride, as was confirmed by spiking the samples with fluoride standard. Additionally, this peak is not visible on the second set of electropherograms, which are for nerve agent VX. On the contrary, a specific, large cationic peak (marked with an asterisk in Fig. 4B) appears at approximately 83 seconds for all matrices contaminated with VX. Heleg-Shabtai et al. [25] have separated VX and its degradation products (EMPA) by CE using a 1 mM carbonate background electrolyte at pH 7.7 with LIF detection. As the pKa of VX is high (the values given in the literature range between 8.6 and 9.4) [58,59], the undegraded VX migrated ahead of the EOF as a cationic peak in their electrolyte system at pH 7.7. The MES/HIS electrolyte used in this work has a pH of 6. Any remaining VX should also be positively charged; we therefore assume that the large positive peak corresponds to unhydrolyzed VX.

Table 2 contains the recovery values for sarin, soman and VX from the different matrices with corresponding concentrations of particular degradation products detected by the portable CE analyser. The results reveal that the lowest recovery (ranging between 0.02 and 0.4%) was obtained from the concrete matrix. This is an obvious consequence of the nature of concrete, which is much more porous than the other solid matrices. In some cases, the recovery rate of the samples obtained from the concrete matrix using the wiping technique is up two orders of magnitude lower than

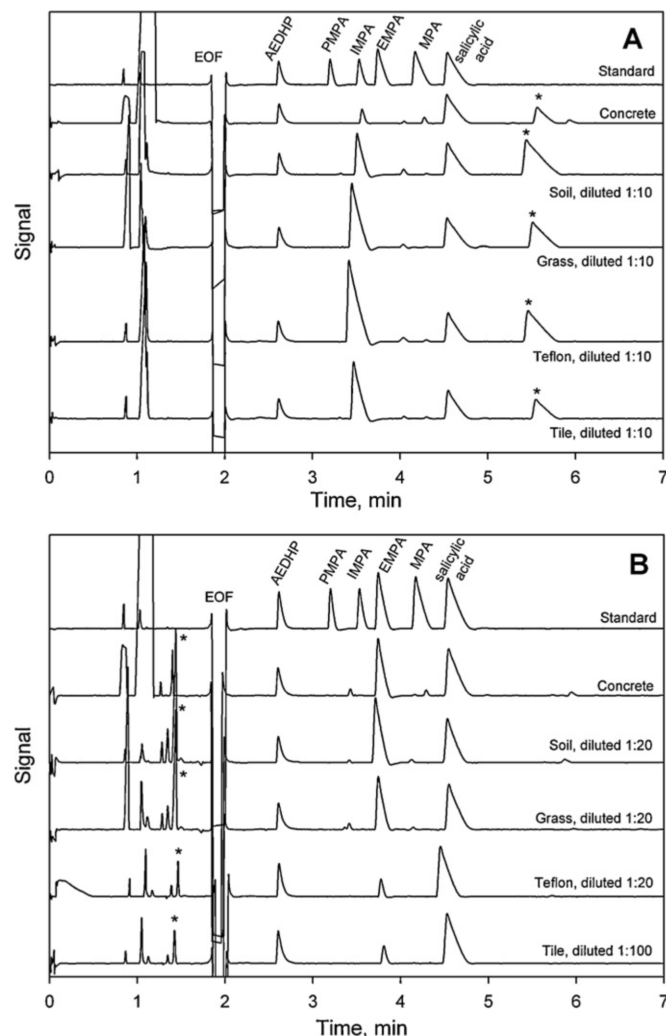

Fig. 4. Separation of hydrolysis products of nerve agents sarin (A) and VX (B) in various matrices. CE conditions: as in Fig. 3.

the recoveries from other matrices. To confirm that the wiping technique is appropriate for concrete samples and that the low recoveries were due to the absorption of the NA into the matrix, control experiments were performed using a different sampling method. The same (previously wiped) concrete samples were put into enclosed containers, covered with 10 mL of deionized water, sonicated for 10 min and the extracts analyzed. Because the recoveries were still much lower than they were from the other matrices even with the sonication approach (by at least one order of magnitude), wipe sampling was deemed to be an appropriate option as it involves less handling and does not require the use of an ultrasound bath. The recoveries for the samples obtained from the other matrices ranged between 4.6–34.5% for Teflon, 2.7–16.7% for tile, 3.2–9.4% for grass and 2.3–8.7% for soil. There was no obvious trend in the behaviour of the various nerve agents on different matrices.

Table 2
Recovery data for sarin, soman and VX on various matrices.

Matrix	sarin				soman				VX			
	Concentration of IMPA, mM		Recovery, %		Concentration of PMPA, mM		Recovery, %		Concentration of EMPA, mM		Recovery, %	
	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h
Soil	1.67	5.43	2.34	7.60	1.25	3.24	2.28	5.91	3.26	1.91	8.72	5.11
Grass	3.73	6.72	5.22	9.42	2.47	2.03	4.51	3.70	2.18	1.19	5.84	3.19
Teflon	5.64	24.62	7.89	34.49	2.55	9.25	4.64	16.84	2.40	2.22	6.43	5.94
Tile	3.16	1.93	4.42	2.71	3.62	9.16	6.60	16.69	2.00	1.39	5.35	3.71
Concrete, wiped	0.04	0.03	0.05	0.04	0.02	0.06	0.03	0.11	0.12	0.15	0.32	0.41
Concrete, sonicated	0.40	0.57	0.56	0.79	0.15	0.42	0.27	0.76	0.85	0.88	2.26	2.35

Table 3
Ratios of NA peak areas after 0.3 and 3 h of application of NA (possible outliers are given in bold italic).

Nerve agent	Matrix				
	Teflon	Tile	Concrete	Soil	Grass
CE: $A_3^{CE}/A_{0.5}^{CE}$					
sarin	4.6	0.6	***0.8***	3.2	1.8
soman	***3.7***	2.5	3.4	3.0	0.8
VX	1.0	0.7	1.3	0.6	0.5
GC–MS: $A_{0.5}^{Chr}/A_3^{Chr}$					
sarin	4.6	0.3	0.1	3.0	1.6
soman	1.8	2.8	3.1	3.7	3.2
VX	0.6	0.3	***3.2***	1.6	1.1
HPLC–MS: $A_{0.5}^{Chr}/A_3^{Chr}$					
sarin	2.3	***97.9***	2.5	2.0	1.6
soman	1.6	1.6	4.0	3.8	1.9
VX	0.9	1.0	1.1	1.1	1.0

In most cases, the recoveries for the soil and vegetation samples were lower than for the solid surface samples, such as tile or Teflon.

3.5. Comparison of CE data with GC–MS and HPLC–MS

To compare the results of the CE analysis with GC–MS and HPLC–MS, the nerve agents were applied to the five matrices, extracted with acetonitrile and immediately analyzed in a laboratory using standard analytical protocols for GC and HPLC determination of the nerve agents and their hydrolysis products. Due to differences in the chemical nature of the extraction solutions applied to the different matrices, direct comparison of CE and chromatographic methods would be difficult. However, the degradation process of NA in different matrices and their extraction should not depend on the analytical method. The NA peak area ratio $A_{0.5}^{Chr}/A_3^{Chr}$ was compared with that of the NA degradation products $A_3^{CE}/A_{0.5}^{CE}$. The indexes *Chr* and *CE* indicate the peak area ratios obtained by the corresponding chromatographic and CE measurements, and 0.5 and 3 denote the duration of exposure to the nerve agents. The data are presented in Table 3. If the nerve agents had decomposed appreciably over time, the numbers in Table 3 (within the margin of experimental error) should be greater than one, which is the case only for the Teflon matrix. Deviations from that premise can be observed for the other matrices, and these may be explained by the nature of a particular matrix and the extent of absorption. Nevertheless, it is still possible to compare the different matrices. With the exception of the obvious outliers (the point was considered an outlier when it was outside the 0.995 confidence band of the corresponding regression line (Student's $t = 3.5$)), the square of correlation coefficients and the regression equation can be calculated from the data represented in Table 3. The results for CE and GC–MS data are as follows:

$$\frac{A_{0.5}^{\text{GC–MS}}}{A_3^{\text{GC–MS}}} = (0.93 \pm 0.13)\frac{A_3^{CE}}{A_{0.5}^{CE}} + (0.2 \pm 0.3); \quad R^2 = 0.89$$

The square of correlation coefficients between CE and HPLC–MS and GC–MS and HPLC–MS were $R^2 = 0.41$ and $R^2 = 0.30$, respectively.

There is a good correlation between the CE and GC–MS data, with a slope close to unity, whereas the correlation between CE and HPLC–MS and between GC–MS and HPLC–MS is nonexistent. This may be a result of further degradation of the NA during HPLC aqueous elution.

Assuming abundant atmospheric humidity at the test site compared to the amount of NA applied to the various matrices, and an irreversible pseudo-first-order reaction of decomposition, the rate of degradation product formation can be estimated from CE measurements using degradation product peak areas from electropherograms recorded at 0.5 and 3 h after the application of the nerve agent to the matrix. However, due to the occurrence of absorption in many of the matrices, meaningful data for Teflon can only be obtained for sarin and soman. The formation rate of IMPA (the degradation product of sarin) was $0.28 \pm 0.02\ \text{h}^{-1}$ and that of PMPA (the degradation product of soman) was $0.5 \pm 0.2\ \text{h}^{-1}$. The data reported in the literature vary significantly. Assuming average values for humidity, rainfall, and solar flux, reported approximate lifetimes under equivalent conditions are 5 h for soman and 30 min for sarin [60], which compare roughly with our data. Since soman is more persistent than sarin, our data indicate that the formation rates of primary degradation products might not give an accurate indication of degradation rates of NA. VX is too persistent to measure its decay in 3 h.

4. Conclusions

This research demonstrates for the first time that a portable CE system with contactless conductometric detection can be used for rapid and accurate identification and quantification of genuine nerve agents that have been deposited on various matrices. The extraction procedures that were developed allow for timely and efficient sampling of nerve agents from contaminated areas and analysis of their degradation products. The entire procedure

including instrument start-up, sampling and analysis takes approximately 10 min, which is the fastest screening with a liquid phase separation method available to date. Three nerve agents (sarin, soman and VX) were positively identified in the five matrices studied, based on hydrolysis product peak identification. Nonexistent or very minor interference from the sample background was observed with the selected BGE and CE polarity, as the major interfering compounds migrate ahead of or behind the degradation products of CWA. Recoveries from different matrices were compared and ranged from 2 to 35% with the exception of the sample taken from concrete, for which the recovery was up to two orders of magnitude lower. The hydrolysis rate constants were estimated for the duplicate samples that were taken at 30 min and 3 h after exposure, and they compare favorably with the data in the literature. The CE data correlated well with the results obtained with the GC–MS reference method. The sampling and analysis techniques described above represent the most rapid method of screening for genuine NA using portable CE instruments that has been presented to date.

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