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High-sensitivity determination of the degradation products of chemical warfare agents by capillary electrophoresis–indirect UV absorbance detection

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Abstract

Capillary electrophoresis coupled with indirect UV absorbance detection was employed for the determination of the chemical warfare agent degradation products: methylphosphonic acid, ethyl methylphosphonate, isopropyl methylphosphonate, and pinacolyl methylphosphonate. Glutamic acid was used as a buffering agent at its isoelectric point (pH 3.22). In its zwitterionic form, glutamic acid does not act as a competing co-anion in the system, thus providing buffering capacity while maintaining high sensitivity. The indirect probe (phenylphosphonic acid) concentration was lowered to 1 m*M* from the 10 m*M* in previous literature studies, further enhancing sensitivity. Detection limits of 2 μ*M* were achieved with hydrodynamic injection and up to 100-fold lower using electrokinetic injection. The increased buffering capacity of this system over previous methods led to migration time reproducibility RSD values of 0.18 to 0.22%. This represents a 10-fold improvement in reproducibility over previous studies with comparable or improved sensitivity.

Keywords: Warfare agents; Phosphonic acids

1. Introduction

Chemical weapons have become a serious threat to society in recent years. The Chemical Weapons Convention (CWC) is an international treaty to eliminate the worldwide threat of chemical warfare. To uphold the regulations set forth by the CWC, it has become essential to develop analytical techniques to monitor chemical warfare (CW) agent use. Fortunately, most CW agents degrade into harmless monoester and alkyl phosphonic acids after relatively short exposure to the environment. For instance, the nerve agent sarin undergoes a rapid hydrolysis to form isopropyl methylphosphonate (IMPA; monoester), which then undergoes a much slower hydrolysis to form methylphosphonic acid (MPA; alkyl). Other nerve agents such as soman and VX degrade through a similar mechanism with MPA being the final product for each, as shown in Fig. 1. Thus these degradation products can be safely analyzed in water or soil samples taken from suspected attack sites.

Capillary electrophoresis (CE) has been shown to be effective for the separation of phosphonic acids [1–8]. Unfortunately, phosphonic acids do not contain a chromophore nor a fluorophore, so detection is problematic. Like carboxylic acids [9], derivatization

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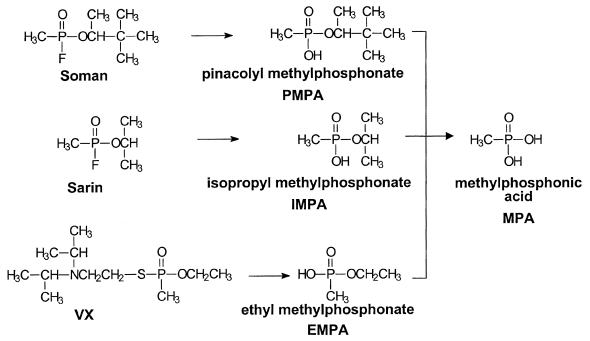

Fig. 1. Degradation pathways of some common nerve agents.

of phosphonic acids is not trivial. In high-performance liquid chromatography (HPLC), labeling of phosphonic acids has been performed with *p*-bromophenacyl bromide for UV absorbance detection [10] and *p*-(9-anthroyloxy)phenacyl bromide for fluorescence detection [11]. These labeling reactions are time consuming (>1 h [10]), require non-aqueous conditions, and produce numerous side-products. Thus these derivatization techniques are not very attractive, and definitely not suitable for portable instruments for monitoring CW agent use in the field.

When compounds are not optically active and are not easily derivatized, indirect detection is sometimes the best alternative. In indirect detection, an absorbing or fluorescing probe is added to the buffer. Displacement of the probe by the analyte produces a decrease in signal [12,13]. Due to its universality and simplicity, indirect UV absorbance detection has been the predominant detection scheme for phosphonic acid analyses by CE [1–4,6–8]. Unfortunately, sensitivity has generally been poor with detection limits in the 10^{-5} *M* range [1–4].

In a recent series of papers, Nassar and co-workers reported excellent sensitivity for phosphonic acids with CE–indirect UV detection [6–8]. Detection limits as low as 10^{-6} *M* using hydrodynamic injection [6,7] and 10^{-8} *M* with electrokinetic injection [8] were achieved. As well as achieving superior detection limits, Nassar et al. also demonstrated sample clean-up techniques for spiked water and soil samples [8]. Despite the success of this method, it suffers from numerous shortcomings.

Firstly, Nassar et al. used 200 m*M* borate to control the pH at 4.0 [8]. This buffer was adapted from earlier work by Pianetti et al. who reported an increase in signal-to-noise ratio on increasing borate concentration in their system while at pH 6 [1]. Borate has a pK_a of 9.27 [14]. Thus it has virtually no buffering capacity at pH 4. Attempts to prepare this buffer in our laboratory proved futile. Great care was required to titrate the solution to near pH 4, but then even a slight dilution of the buffer with water (i.e., filling the volumetric flask to the mark) resulted in an increase in the pH to as high as 4.5. Any buffering capacity of the system would most likely originate from the probe, phenylphosphonic acid (pK_{a1}=1.83, pK_{a2}=7.07 [15]). Such low buffering capacity is known to lead to irreproducible data in CE. In a recent head-to-head comparison of a buffered and an unbuffered chromate electrolyte, both systems yielded similar selectivity, efficiency, and sensitivity, but the buffered electrolyte exhibited far superior reproducibility of migration times and peak areas [16].

Secondly, all previous methods for indirect UV detection of phosphonic acids have used a relatively high probe concentration of 10 m*M* [1,8]. In indirect detection, the limit of detection (LOD) is given by [17]:

$$C_{\mathrm{LOD}} = \frac{C_{\mathrm{P}}}{T_{\mathrm{R}} \cdot \mathrm{DR}} \qquad (1)$$

where C_P is the concentration of the probe, T_R is the transfer ratio (the number of probe molecules displaced by one analyte molecule), and DR is the dynamic reserve (ratio of the background absorbance to the noise). Thus the relatively high probe concentration may have been limiting sensitivity.

In the present work, we demonstrate a very robust and highly sensitive method for detection of phosphonic acids by CE–indirect UV detection. Specifically, we employ a zwitterionic buffering agent that provides adequate buffering at our choice of pH without introducing a competing co-anion into the buffer [17]. Further, we are able to reduce the probe concentration by manipulating the effects of system peaks. This is accomplished through employing an alternative electroosmotic flow (EOF) modifier.

2. Experimental

2.1. Apparatus

A P/ACE MDQ (Beckman Instruments, Fullerton, CA, USA) equipped with a UV absorbance detector was used for all experiments. Data acquisition and control was performed using P/ACE Station software (Beckman) for Windows 95 on a Pentium personal computer. Untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 μm, an outer diameter of 365 μm, and a total length of 50 cm (40 cm to the detector) were used unless otherwise specified. In all experiments, the capillary was thermostated at 25°C.

2.2. Reagents

All solutions were prepared in Nanopure 18 MΩ water (Barnstead). Buffers were prepared from L-glutamic acid (Aldrich) and the pH was adjusted by titration with NaOH (BDH). The indirect detection probe was phenylphosphonic acid (Aldrich), and was used as received. Buffers were prepared daily by titrating a solution of the buffering agent and the probe to the appropriate pH, followed by addition of the surfactant. Methylphosphonic acid (MPA), ethyl methylphosphonate (EMPA), and pinacolyl methylphosphonate (PMPA) were obtained from Aldrich while isopropyl methylphosphonate (IMPA) was synthesized at the Defense Research Establishment at Suffield (Suffield, AB, Canada). The double-chained surfactant didodecyldimethylammonium bromide (DDAB; Aldrich) and the zwitterionic surfactant surfactant coco(amidopropyl)ammoniumdimethylsulfobetaine [$RCONH(CH_2)_2N^+(CH_3)_2CH_2$-$CH(OH)CH_2SO_3^-$, $R=C_8-C_{18}$] (Rewoteric AM CAS U; Witco) were used as received. Samples of the surfactant CAS-U can be obtained from the authors upon request.

2.3. Separations

Each new capillary was pretreated with 0.1 *M* NaOH for 10 min followed by water for 5 min. Employing DDAB as an EOF modifier, a 0.1 m*M* solution in water was rinsed through the capillary at 20 p.s.i. for 3 min, followed by a 1-min rinse with the separation buffer (1 p.s.i. = 6894.76 Pa). Employing CAS-U as an EOF modifier, buffer containing 1 m*M* CAS-U was flushed through the capillary for 3 min at 20 p.s.i. and the surfactant remained in the buffer for the separation. A 3-s hydrodynamic injection (0.5 p.s.i.) was used for all preliminary and optimization experiments while a 10-s injection (0.5 p.s.i.) was used for detection limits and reproducibility studies. Separations were performed under an electric field strength of −400 V/cm (−20 kV over 50 cm) with a voltage ramp time of 0.5 min, unless otherwise indicated. Indirect UV detection at 214 nm was used with a data acquisition rate of 8 Hz.

The optimum buffer for the CW agent degradation products consisted of 10 m*M* glutamic acid, 1 m*M* phenylphosphonic acid, and 1 m*M* CAS-U at pH 3.22. Optimized separations employed a 10-s hydrodynamic injection (0.5 p.s.i.) and an applied potential of −20 kV.

3. Results and discussion

3.1. Optimization of probe concentration

As mentioned above, reduction of the probe concentration in indirect detection generally results in sensitivity gains. Thus, reducing the probe concentration from the standard 10 m*M* phenylphosphonic acid [1,6] was a major goal of this work. As simple as this may sound, this proved to be a rather challenging endeavor.

Since phosphonic acids are negatively charged it is desirable to reverse the direction of the EOF to reduce the analysis time. The double-chained cationic surfactant DDAB has been successfully employed for EOF reversal for phosphonic acid analyses [6]. DDAB has proven to be effective under acidic conditions [18] and adheres to the capillary so strongly that excess surfactant can be flushed from the capillary after an initial coating [19]. In contrast, traditional single-chained surfactants such as cetyltrimethylammonium bromide must be present in the buffer at relatively high concentrations to maintain an equilibrium between free surfactant molecules and those adsorbed at the wall (dynamic coating) [19].

Thus DDAB is attractive for anion analyses, as ion-pairing between the anion and the cationic surfactants and micelles in the buffer can be avoided.

Fig. 2 illustrates the effect of reducing the probe concentration on the separation of the four CW agent degradation products using DDAB for EOF reversal. At a phenylphosphonic acid concentration of 10 m*M*, baseline resolution is achieved for all four compounds and there is adequate spacing between the PMPA peak (4) and the system peak (S). At 5 m*M*, resolution between MPA and EMPA decreases and the system peak is merging with the PMPA peak. Finally, the system peak co-migrates with PMPA at 1 m*M* phenylphosphonic acid. The peak migration time shifts evident in Fig. 2 are caused by an increase in the reversed EOF as the probe concentration is lowered. As can be seen by the water peak in Fig. 2, the EOF increases from $-4.8\cdot10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ in the presence of 10 m*M* phenylphosphonic acid to $-7.8\cdot10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ with 1 m*M* phenylphosphonic acid. The slower EOF at higher probe concentrations is most likely due to partitioning of the probe into the cationic bilayer at the capillary wall. The negatively charged probe will in effect dilute the charge on the cationic wall coating, thus slowing the reversed EOF. Attempts to shift the system peak to longer migration times to eliminate co-migration with the analytes proved unsuccessful. Further investigations of a number of different probes such as mono- and disulfonated benzenes and naphthalenes at concentrations of ≤ 1 m*M* using DDAB were similarly unsuccessful. These probes all exhibited their own distinct set of system peaks or baseline anomalies and provided no obvious advantage over the traditional phenylphosphonic acid. These results are consistent with pioneering work by Pianetti et al. who found phenylphosphonic acid to be the most suitable probe for this set of analytes [1].

Since the system peak issue at low probe concentrations with DDAB appeared to relate to the EOF and thus the wall coating, an alternate surfactant system was employed to control the EOF. The zwitterionic surfactant CAS-U has been previously employed for EOF suppression in anion analyses [20] and for prevention of protein adsorption in CE [21]. The zwitterionic surfactant produces a very suppressed EOF in either the forward or reverse direction depending on the buffer conditions [22]. Further, employing CAS-U in this study should reduce ion-pair interactions between the surfactant and the analyte or probe.

Fig. 3 shows the separation of the four CW agent degradation products using 1 m*M* phenylphosphonic

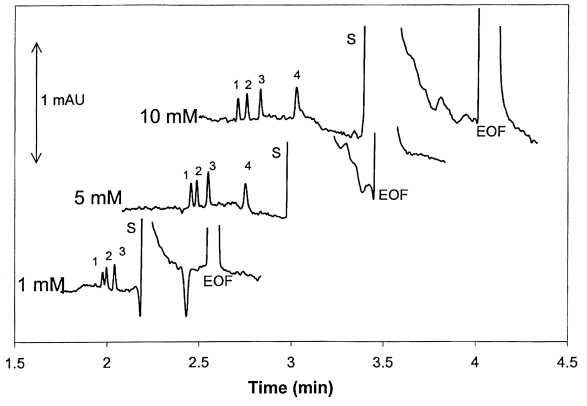

Fig. 2. Effect of phenylphosphonic acid (probe) concentration on the separation of CW agent degradation products using DDAB for EOF reversal. Peaks: (1) MPA; (2) EMPA; (3) IMPA; (4) PMPA; (S) system peak; (EOF) water plug. Experimental conditions: buffer: 10 m*M* glutamic acid, pH 3.22, 1–10 m*M* phenylphosphonic acid; 3-s hydrodyanmic injection at 0.5 p.s.i.; −15 kV applied potential; detection at 214 nm; 10 μ*M* phosphonic acid mixture shown.

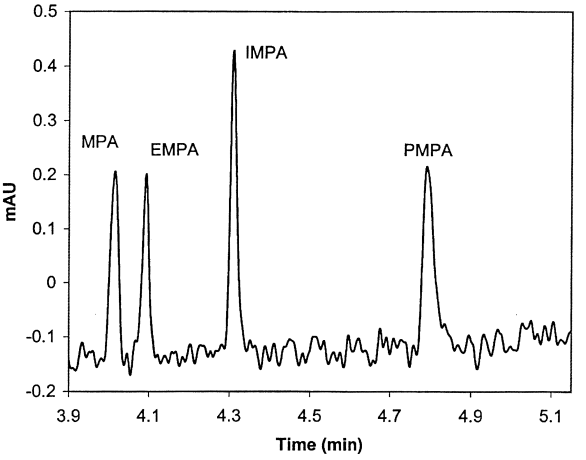

Fig. 3. Separation of CW agent degradation products using CAS-U for EOF suppression. Experimental conditions: buffer: 10 m*M* glutamic acid, pH 3.22, 1 m*M* phenylphosphonic acid, 1 m*M* CAS-U; 10-s hydrodynamic injection at 0.5 p.s.i.; −20 kV applied potential; detection at 214 nm; 10 μ*M* phosphonic acid mixture shown.

acid and CAS-U for EOF suppression. Adequate spacing between the system peak and PMPA was achieved, although the separation time was lengthened to 5 min. The EOF generated by this buffer system was measured to be low and in the reverse direction ($-1.5\cdot10^{-4}$ cm^2 V^{-1} s^{-1}), which is advantageous for this work. At concentrations below 1 m*M* phenylphosphonic acid, the system peak and PMPA peak merged. Nevertheless, a 1 m*M* phenylphosphonic acid concentration represents a 10-fold decrease in the probe concentration compared to previous methods, and theoretically a 10-fold improvement in sensitivity based on Eq. (1).

3.2. Choice of buffering agent

Buffering of electrolytes for indirect detection while maintaining high-sensitivity is a formidable task. In the case of anion analysis, introduction of a buffering co-anion into the system (i.e., phosphate, acetate, etc.) will lead to competitive displacement of the co-anions and the probe by the analyte [17]. This will decrease the transfer ratio T_R in Eq. (1) and thereby compromise sensitivity. Techniques to overcome this problem have included employing a low mobility buffering agent [23] or titrating the acid form of a probe with a buffering base to near the pK_a of the base [16].

Another alternative to buffering electrolytes for indirect detection is the use of amphoteric electrolytes, introduced by Doble et al. [17]. At its isoelectric point (p*I*), an ampholyte exists in a zwitterionic form with a net charge of zero. An ampholyte will buffer at its p*I* when the pK_a values of the two buffering groups are roughly within 1 unit of the p*I* [17]. For instance, the ampholyte glutamic acid has two carboxylic acid groups with pK_a values of 2.19 and 4.25 and an amino group with pK_a 9.67. With a p*I* of 3.22, the carboxylate groups of glutamic acid provide adequate buffering capacity at its p*I*. With zero net charge, such ampholytes are ideal for indirect detection since they will not lead to competitive displacement of the probe. Thus, the transfer ratio for indirect detection remains high. Further, zwitterionic buffers do not add substantially to the conductivity of the electrolyte and may be added in relatively large quantities to provide adequate buffering.

For analyses of phosphonic acid by CE, acidic electrolytes are desirable. Phosphonic acids are relatively strong acids with pK_a ranging from ~1.7 to 2.4 and are therefore highly negatively charged at pH>3. Nassar et al. reported optimum selectivity between the phosphonic acids at pH 4 [6]. A further advantage of working under acidic conditions are that potential interfering ions such as carbonates and carboxylates will be largely protonated and thus will not co-migrate with the phosphonic acids.

In this work, we tested two zwitterionic buffer systems: aspartic acid (p*I* 2.77) and glutamic acid (p*I* 3.22). Using aspartic acid at pH 2.77, baseline resolution between MPA and EMPA could not be achieved. With pK_{a1} of 2.4, MPA is partially protonated at pH 2.77. Thus its mobility is reduced and it co-migrates with the stronger acid EMPA (pK_a 1.7). Employing glutamic acid as a buffering agent at pH 3.22, baseline resolution of all phosphonic acids was achieved, as shown in Fig. 3. Thus 10 m*M* glutamic acid was chosen as the buffering agent. This concentration was sufficient to provide adequate buffering as demonstrated previously by Doble et al. [17] and by the excellent reproducibility achieved in this study (see below).

3.3. Calibration, detection limits, and reproducibility

Quantification studies were performed only for MPA to avoid the possible breakdown of the other CW agent products (see Fig. 1). Given, the universal nature of indirect detection, similar sensitivities are expected for the other CW agent products. Using the optimum buffer conditions (Section 2.3 and Fig. 3), linear calibration was achieved for MPA over a range of 5–100 μ*M* (slope of log response vs. log concentration plot = 1.08 ± 0.04). Detection limits were calculated according to the method specified by the US Environmental Protection Agency [24]. Using this procedure a solution containing analyte at one to five times the approximate detection limit is run in replicate ($n \geq 7$). The limit of detection (LOD) is then determined by:

$$C_{LOD} = s \cdot t_{n-1} \quad (2)$$

where *s* is the standard deviation of the calculated concentrations and t_{n-1} is the *t*-statistic (one-sided)

at the 99% confidence level with $n-1$ degrees of freedom. In this case, a sample containing 10 μM MPA was run 10 times and quantified, yielding a limit of detection of 2 μM ($s=0.73$ and $t=2.821$). This LOD is roughly a 10-fold improvement over that achieved by Pianetti et al. [1] and comparable to that achieved by Nassar et al. [6]. Employing electrokinetic injection (−10 kV for 10 s), a sensitivity enhancement of ~100-fold was achieved. These results are comparable with those of Nassar et al. [8]. Fig. 4 compares the detector response for MPA using hydrodynamic and electrokinetic injection.

Reproducibility data was obtained over 30 consecutive runs with no capillary conditioning between runs (just a 3-min buffer rinse). Excellent migration time reproducibility was achieved, with RSD values ranging from 0.18 to 0.22% for the four analytes studied. This is substantially better than that achieved in the previous work by Pianetti et al. (<1.8%) [1] and Nassar et al. (4–5%) [7]. This excellent migration time reproducibility is comparable to the 0.2–0.8% RSD achieved by Doble et al. for five anions using a 10 mM glutamic acid buffer at pH 3.22 [17]. Peak area reproducibility was also respectable with RSD values of <10% at a concentration of 10 μM (~five times the LOD).

The effect of possible interfering ions (i.e., Cl^-, SO_4^{2-}, etc.) was not investigated in this study. These high-mobility ions will migrate much faster than the phosphonic acids and thus pose no threat to interfere. However, to achieve maximum sensitivity employing electrokinetic injection, sample conductivity must be kept at a minimal [25]. In such a case, sample clean-up procedures can be easily performed [8].

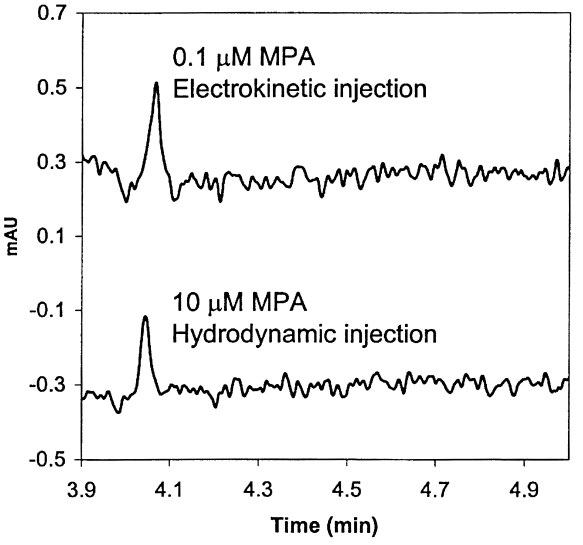

Fig. 4. Detector response for MPA using hydrodynamic or electrokinetic injection. Hydrodynamic injection: 10 s at 0.5 p.s.i. (10 μM MPA shown). Electrokinetic injection: 10 s at −10 kV (0.1 μM MPA shown). Experimental conditions as in Fig. 3.

4. Conclusions

The methodology described above represents a simple, robust, and sensitive technique for the determination of chemical warfare agent degradation products by capillary electrophoresis. The zwitterionic buffer system employed unquestionably leads to greater reproducibility while maintaining high sensitivity.

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