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Field-amplified sample stacking for the detection of chemical warfare agent degradation products in low-conductivity matrices by capillary electrophoresis-mass spectrometry

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Abstract

Preconcentration of chemical warfare agent degradation products (alkylphosphonic acids and alkyl alkylphosphonic acids) in low-conductivity matrices (purified water, tap water and local river water) by field-amplified sample stacking (FASS) was developed for capillary electrophoresis (CE) coupled to ion trap mass spectrometry. FASS was performed by adding a mixture of $HCOONH_4$ and NH_4OH in appropriate concentrations to the sample. This allowed to control the conductivity and the pH of the sample in order to obtain FASS performances that are independent of analyte concentration. The influence of different parameters on FASS (sample to background electrolyte (BGE) conductivity ratio, injection volume and concentration of BGE) was studied to determine the optimal conditions and was rationalized by using the theoretical model developed by Burgi and Chien. A good correlation was obtained between the bulk electroosmotic velocity predicted by this model and the experimental value deduced from the migration time of the electroosmotic flow marker detected by mass spectrometry (MS). This newly developed method was successfully applied to the analysis of tap water and local river water fortified with the analytes and provided a 10-fold sensitivity enhancement in comparison to the signal obtained without preconcentration procedure. The quite satisfactory repeatability and linearity for peak areas obtained in the 0.5–5 $\mu g\,mL^{-1}$ concentration range allow quantitative analysis to be implemented. Limits of detection of 0.25–0.5 $\mu g\,mL^{-1}$ for the alkyl alkylphosphonic acids and of 0.35–5 $\mu g\,mL^{-1}$ for the alkylphosphonic acids were reached in tap water and river water.

Keywords: Alkylphosphonic acids; Capillary electrophoresis-mass spectrometry (CE-MS); Chemical warfare agent degradation products; Environmental matrices; Field-amplified sample stacking

1. Introduction

Alkyl alkylphosphonic acids (R_1–P(O)(OH)(OR_2)) and alkylphosphonic acids (R_1–P(O)$(OH)_2$) result from the hydrolysis of G-type and V-type nerve agents such as tabun, sarin, soman and VX over time. These nerve agent degradation products are subjected to the Chemical Weapons Convention (CWC) [1] which entered into force in April 1997. The Organization for the Prohibition of Chemical Weapons (OPCW) ensures the compliance with the CWC by proceeding to inspections, on-site analysis and sampling followed by off-site analysis that can be performed by a network of expert laboratories which are regularly subjected to OPCW proficiency tests. The most widely used separation techniques that are employed for the determination of chemical warfare agents and their degradation products are gas chromatography and liquid chromatography [2]. Although less used, capillary electrophoresis (CE) displays specific interests for the analysis of chemicals related to the CWC thanks to its intrinsic capabilities for the analysis of polar compounds such as phosphonic acids, its high-separation efficiency, very low-sample consumption and separation principles which are very different, and consequently very complementary to those of chromatographic techniques. Several articles have thus reported the use of CE for the analysis of nerve

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agent degradation products [3]. Because these compounds are generally devoid of chromogenic groups, they have often been detected using indirect UV detection [4–11]. However, the sensitivity of conventional UV–VIS detection is limited by the very short optical path length corresponding to the small inner diameter of the capillary (25–100 μm). Consequently, more sensitive detection techniques have been used for the detection of nerve agent degradation products such as conductivity [6,7,12], laser-induced fluorescence [13,14], flame photometric detection [15,16] and mass spectrometry (MS) [17–19]. The latter is particularly interesting because it provides very powerful identification capabilities. The great complementary of CE and MS for the identification of chemicals related to the CWC was reported in our previous works [20,21].

Sensitivity can also be improved by using in-capillary preconcentration techniques which allows to increase the injected sample volume. This volume is usually limited to ca. 1% of the capillary volume in the absence of preconcentration procedures to avoid resolution loss and peak shape alteration. The different preconcentration techniques that can be used in CE are widely described in the literature [22–26] and generally rely on the same principle, namely the reduction of a large injected sample plug into narrow and concentrated zones of analytes. The simplest preconcentration technique is field-amplified sample stacking (FASS) which only requires the use of a background electrolyte (BGE) of higher conductivity than the sample. The fact that local electric field is inversely proportional to conductivity results in a high-electric field in the hydrodynamically injected sample zone, providing a high-analyte velocity in this low-conductivity region. Consequently, when analytes penetrate into the low-electric field of the BGE zone, they slow down and are concentrated into narrow bands. The conductivity ratio between the BGE and the sample (γ ratio) strongly influences the performances of FASS with respect to sensitivity enhancement and resolution.

In a previous article, we described the development and optimization of a capillary electrophoresis-mass spectrometry (CE-MS) method for the detection and identification of chemical warfare agent degradation products (alkyl alkylphosphonic acids and alkylphosphonic acids) in pure water and soil extracts [20]. Good identification capabilities were obtained, particularly when using CE-MS–MS for the identification of co-migrating isomeric alkyl alkylphosphonic acids, but the sensitivity was limited by the low-injected sample amount and the relatively low-ionization efficiency obtained for analytes of low-*m*/*z* ratios (ethylphosphonic acid and methylphosphonic acid). Thus, a brief investigation of FASS was undertaken for the preconcentration of alkyl alkylphosphonic and alkylphosphonic acids in water prior to CE-MS [21]. This approach, however, presented two drawbacks. First, the important difference between the pH of the sample (about 4) and that of the BGE (8.8) led to a detrimental mismatch of the electroosmotic velocities between these two zones, thus limiting the injected volume to ca. 5% of the capillary volume. Secondly, the conductivity and the pH of the sample depended on analyte concentration. This induced a variation of the γ ratio and consequently of FASS performances with analyte concentration, which is incompatible with quantitative analysis. In the present work, a more detailed investigation of various parameters (BGE composition, γ ratio, injection volume) has been performed, so that overall FASS performances can be controlled independently of analyte concentration, in compliance with quantitative analysis requirements. Finally, this newly developed method was successfully applied to environmental low-conductivity matrices (local tap and river waters).

2. Experimental

2.1. Chemicals and sample solutions

Methylphosphonic acid (MPA, purity ≥98%), ethylphosphonic acid (EPA, purity ≥98%), propylphosphonic acid (PrPA, purity ≥95%), phenylphosphonic acid (PhPA, purity ≥98%) and ethyl methylphosphonic acid (EMPA, purity ≥98%) were purchased from Aldrich (St. Quentin Fallavier, France). Isopropylphosphonic acid (IPA), methyl ethylphosphonic acid (MEPA), ethyl ethylphosphonic acid (EEPA), methyl propylphosphonic acid (MPrPA) and propyl methylphosphonic acid (PrMPA) were synthesized at the Centre d'Etudes du Bouchet and were ≥98% purity (^{31}P NMR). The structures of the analytes are reported in Table 1. Ammonium formate (purity ≥99%) was obtained from Fluka (Buchs, Switzerland). Formamide and anhydrous D(+)-glucose used as electroosmotic flow markers were purchased from Aldrich. Methanol (HPLC grade), 25% (w/w) ammonium hydroxide solution and 1 M sodium hydroxide were from Merck (Darmstadt, Germany). Water used throughout was obtained from an alpha-Q system (Millipore, St. Quentin en Yvelines, France). Stock solutions of phosphonic acids were prepared by diluting the compounds in water at a concentration of 10 mg mL^{-1}. The samples analyzed by CE-MS were prepared from these stock solutions diluted in the different studied matrices (purified water, tap water, local river water) to obtain final concentrations of analytes ranging from 0.5 to 5 μg mL^{-1}. The

Table 1
Structures of the studied alkyl- (R_1–P(O)$(OH)_2$) and alkyl alkyl- (R_1–P(O)(OH)(OR_2)) phosphonic acids, migration orders and mass-to-charge ratios of the corresponding pseudo-molecular ions and main fragment ions observed on mass spectra in negative ion mode

	EEPA, peak 1	MPrPA, peak 2	PrMPA, peak 3	MEPA, peak 4	EMPA, peak 5	IPA, peak 6	PrPA, peak 7	EPA, peak 8	PhPA, peak 9	MPA, peak 10
R_1	C_2H_5	$CH_3(CH_2)_2$	CH_3	C_2H_5	CH_3	$(CH_3)_2CH$	$CH_3(CH_2)_2$	C_2H_5	C_6H_5	CH_3
R_2	C_2H_5	CH_3	$CH_3(CH_2)_2$	CH_3	C_2H_5	H	H	H	H	H
M_w (g mol^{-1})	138	138	138	124	124	124	124	110	158	96
$[M-H]^-$ (*m*/*z*)	137	137	137	123	123	123	123	109	157	95
Main fragment ion (*m*/*z*)	109	105	95	91	95	79	79	79	79	79