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Short communication

Separation of sulfur containing chemical warfare related compounds in aqueous samples by micellar electrokinetic chromatography

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Abstract

A method is described in which micellar electrokinetic chromatography (MEKC) is used to separate thiodiglycol, 2,2′-sulfinyldiethanol, 1,4-dithiane, 1,4-thioxane, O-isobutyl methylphosphonothioic acid and O-ethyl methylphosphonothioic acid in aqueous samples. Detection limits range from 1 to 10 μg/ml and the calibration curves are linear over two orders of magnitude. The compounds are separated in under 10 min. The method fulfills our requirements for a rapid, on-site screening technique for these compounds.

1. Introduction

A treaty to ban the use, production and storage of chemical warfare (CW) agents is currently under review by many countries [1]. The treaty calls for destruction of all stockpiles within ten years of ratification. There are also provisions within the treaty for challenge inspections including allegations of use, production, storage and monitoring of destruction. These provisions will require on-site analytical methods for the verification of treaty-related activities. Methods for the detection of CW agents, precursors, manufacturing byproducts and degradation products will be required. Several of these compounds, thiodiglycol, 2,2′-sulfinyldiethanol, 1,4-dithiane, 1,4-thioxane, O-isobutyl methylphosphonothioic acid and O-ethyl methylphosphonothioic acid, contain a sulfur atom which makes them amenable to direct detection with UV spectroscopy.

Our current on-site method [2] for the detection of these compounds in aqueous samples uses a methylene chloride extraction followed by a concentration step or solid-phase extraction/elution and derivitization. The samples are then analyzed by gas chromatography–mass spectrometry (GC–MS). This preparation and analysis are time-consuming. The current goal of our research is to be able to identify these compounds in aqueous samples at a level of 10 μg/ml. Quantitation is not a requirement for on-site analysis, but is used in other work being conducted in our laboratory. One focus of our research is to develop on-site screening methods which are fast and require little sample prepara-

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tion. Screening methods alone do not provide definitive compound identification which is a requirement for treaty-related inspections. During an inspection, time is the limiting factor. Screening methods are used to prioritze samples for further analysis by GC–MS. Among the options being explored are ion chromatography, micro high-performance liquid chromatography and capillary electrophoresis. Capillary electrophoresis is unique because it requires little sample volume, produces fast, high efficiency separations, is flexible in terms of various types of separation techniques which can be performed and used to identify different types of compounds (e.g. free zone, gel and MEKC), generates little waste and can be easily transported. The use of capillary electrophoresis for the separation of some CW-related compounds has been reported [3–5].

MEKC was first described by Terabe and coworkers [6–8]. MEKC is a branch of capillary electrophoresis in which a surfactant is added, above its critical micelle concentration, to the running buffer. The formation of micelles allows the separation of both neutral and charged species. Compounds partition between the micelles and the buffer solution contained within a capillary column. Negatively charged micelles migrate towards the anode under the influence of an electric field which is produced when a voltage is applied across the capillary. The bulk flow, called the electroosmotic flow (EOF), is a consequence of surface charge on the interior wall of the capillary. The direction of the EOF is towards the cathode and is generally greater than the migration velocity of the micelles. Therefore, the net movement of species is towards the cathode. Migration times for neutral species are based upon the amount of time analytes spend partitioned into the micelles. Recent reviews of MEKC have been published [9–11].

This study focuses on the application of MEKC to the separation of thiodiglycol (TDG), 2,2′-sulfinyldiethanol (TDGO), 1,4-dithiane, 1,4-thioxane, O-isobutyl methylphosphonothioic acid (IBMPSA) and O-ethyl methylphosphonothioic acid (EMPSA) in aqueous samples with direct UV detection.

2. Experimental

2.1. Chemicals

Boric acid, β-cyclodextrin, acetonitrile, monobasic sodium phosphate, methanol, thiodiglycol, 1,4-dithiane, 1,4-thioxane and 2,2′-sulfinyldiethanol were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Sodium dodecylsulfate (SDS) was obtained from Fluka (Ronkonkoma, NY, USA). O-Isobutyl methyl-phosphonothioic acid and O-ethyl methylphosphonothioic acid were obtained from Edgewood Research, Development and Engineering Center (Edgewood, MD, USA). Distilled, deionized water (18 MΩ was generated internally from a Barnstead (Dubuque, IA, USA) Nanopure system.

2.2. Apparatus

MEKC experiments were performed using a P/ACE 2100 system (Beckman, Columbia, MD, USA), at 15 kV. The temperature was thermostated at 25°C. A fused-silica capillary with an effective length of 50 cm, total length 57 cm, and 75 μm I.D. was used for all separations. Injections were hydrodynamic for 3 s. Detection was by a single-wavelength UV (deuterium lamp) detector set at 200 nm.

2.3. Procedures

The most significant separation problem was the resolution of the closely related species, TDG and TDGO. Composition of the buffer system was optimized in terms of concentration of SDS, borate, organic modifier (methanol), cyclodextrin and use of another buffer system, phosphate, in order to achieve baseline resolution and best detection of these components. The initial buffer composition chosen was 10 m*M* borate and 50 m*M* SDS at pH 9. The buffer was prepared by dissolution of boric acid and SDS in 18 MΩ, distilled, deionized water. The pH of the solution was adjusted to 9 with 10% (w/w) NaOH.

3. Results and discussion

In order to optimize the separation of TDG and TDGO, we added methanol as an organic modifier. Kaneta et al. [12] showed that acetonitrile and dimethylformamide were effective modifiers for improving the resolution of hydrophobic species, while methanol was better for less hydrophobic compounds. Methanol increases the viscosity of the solution, thus slowing the electroosmotic flow and extending the elution range of the buffer system. Janini et al. [13] showed that the electroosmotic mobility is mainly influenced by buffer viscosity and that other factors such as dielectric constant, zeta potential, and modifier–capillary wall interactions are significant at trace levels of modifier, but diminish as the concentration of modifier increases. In our system, we found that as the methanol concentration increased, the elution times for TDG and TDGO also increased, as expected, but the resolution decreased. The addition of the organic modifier not only affects the elution range, but can also alter the distribution of analytes between the micelles and buffer solution. The addition of MeOH to our system enhances the concentration of the analytes in the buffer relative to the micelles thereby lowering the resolution. A more detailed discussion of the addition of organic modifiers is given in Refs. [12,13].

The addition of other modifiers, such as acetonitrile and β-cyclodextrin, did not enhance the resolution or detection of these compounds.

The last variable changed was the concentration of the surfactant. The optimum resolution occured at an SDS concentration of 100 m*M*. Concentrations above 125 m*M* SDS produced too high a current which leads to increased Joule heating.

The use of phosphate (pH 7, 100 m*M* SDS, 10 m*M* phosphate) as the buffer did not enhance

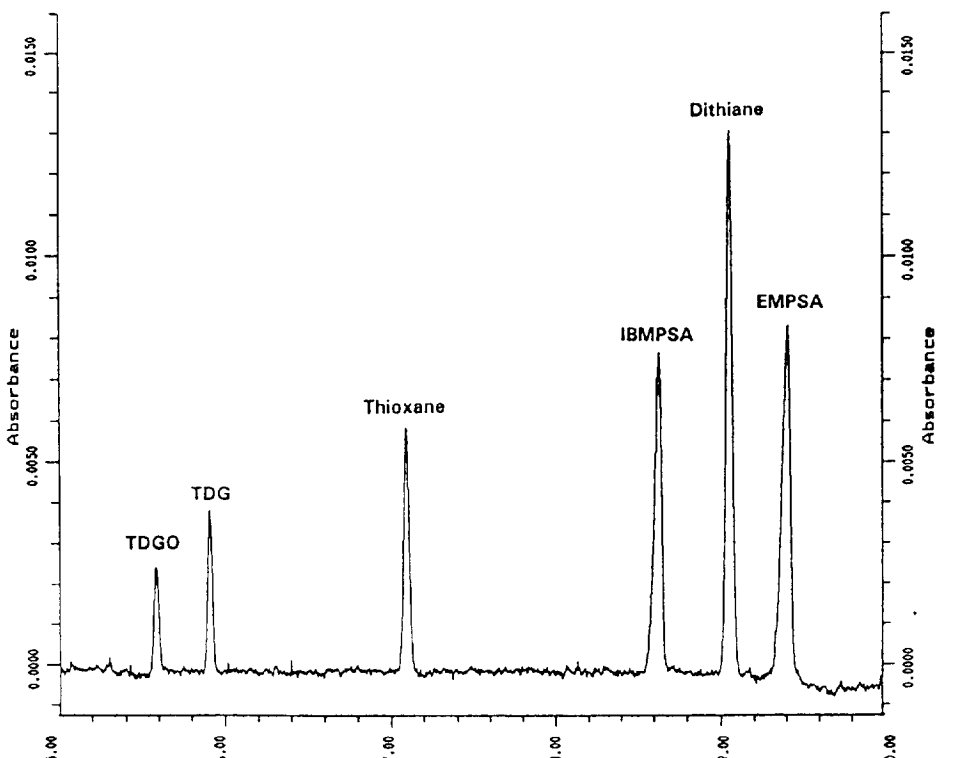

Fig. 1. Electropherogram of the six analytes at 50 μg/ml. Buffer is 10 m*M* borate/100 m*M* SDS at pH 9.0. Separation at 25 kV and 25°C.

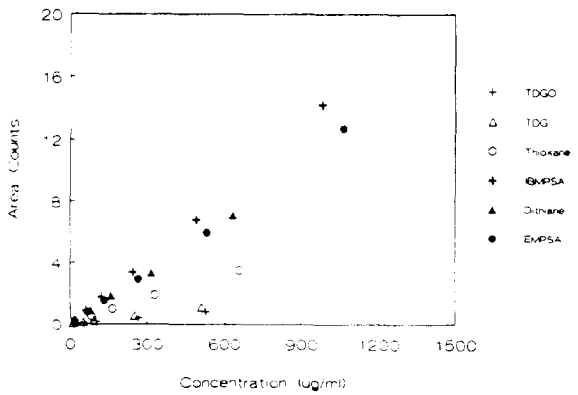

Fig. 2. Calibration curves for the six analytes.

the separation and led to a decrease in the response of the analytes.

An electropherogram of the six compounds of interest is shown in Fig. 1. The compounds are all baseline resolved. Calibration curves for the six compounds are shown in Fig. 2. The plots are linear over two orders of magnitude with correlation coefficients of 0.999 or better. The precision of the method, in terms of area count and migration time reproducibility, along with limits of detection are given in Table 1. The precision for migration times and area counts are all less than 5% except for IBMPSA which is 6.6% (area count). The detection limits, based on a signal-to-noise ratio of 3:1, are well within our goal of 1–10 ppm for this method.

The separation of the neutral species is based on a partitioning between the micelles and the buffer solution. Those compounds which are most hydrophobic spend more time in the micelles. The negatively charged micelles migrate towards the anode against the EOF, however, their net movement is towards the cathode since the magnitude of the EOF is greater than their mobility. Therefore, the more hydrophobic the compound, the longer the migration time. This is shown in Fig. 1 as the order of elution for the neutral species is TDGO, TDG, 1,4-thioxane, and 1,4-dithiane. The separation of ionic compounds, like IBMPSA and EMPSA, is based on a combination of free zone and micellar mechanisms [9].

Environmental samples were collected from the Edgewood Area of Aberdeen Proving Ground, MD, USA. They were spiked and run by MEKC. Fig. 3 shows typical electropherograms of a blank and a spiked sample. All six compounds are easily identified.

4. Conclusion

Micellar electrokinetic chromatography with UV detection serves as a useful screening method for the separation of sulfur-containing chemical warfare related compounds. The method is rapid and sensitive (meets our requirement of 10 μg/ml), requires little or no sample preparation, uses little sample volume and generates minimal waste. The method could easily be adapted to on-site analysis. Currently, we are using this method in the laboratory to screen aqueous samples and aqueous extracts of soils and other

Table 1
Statistical data

Compound	Correlation coefficient	Detection limit (μg/ml)	Precision[a] (area count)	Precision[a] (migration time)
TDGO	0.9999	10	3.2	0.21
TDG	0.9999	10	2.9	0.26
Thioxane	0.9985	8.0	3.6	0.18
IBMPSA	0.9997	1.7	6.6	0.24
Dithiane	0.9992	4.1	4.2	2.5
EMPSA	0.9995	2.5	3.8	2.7

[a] $n = 5$.

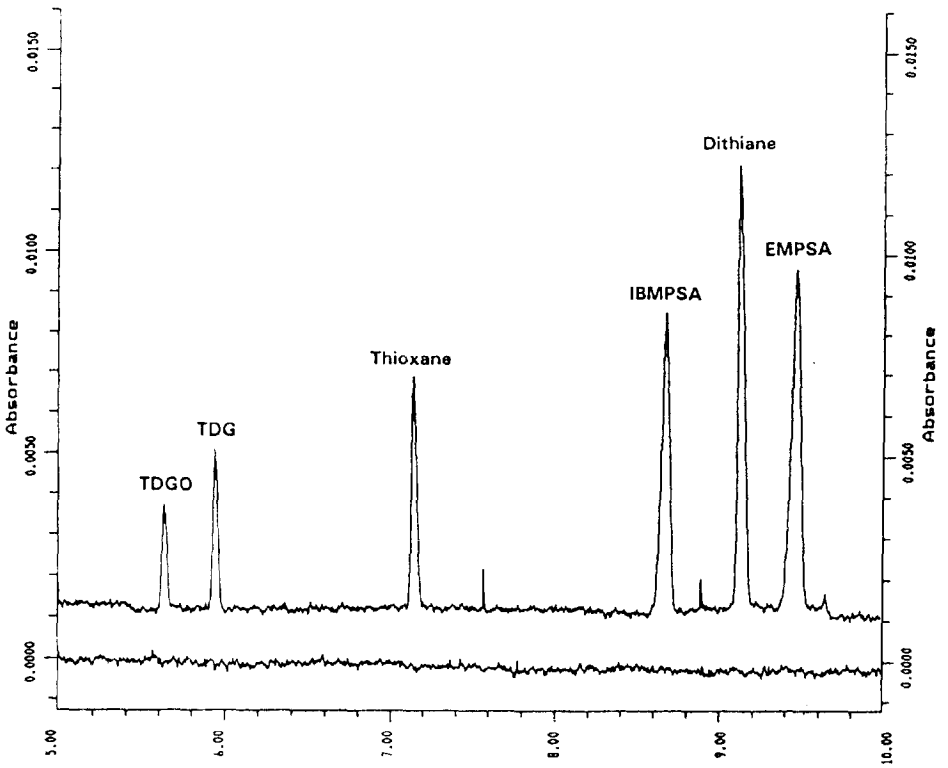

Fig. 3. Environmental water sample analysis using MEKC with sample blank (lower) and laboratory spiked sample (upper). Buffer is 10 m*M* borate 100 m*M* SDS at pH 9.0. Separation at 25 kV and 25°C.

solid samples for these compounds. Future research will be on making our CE instrumentation field portable.

Acknowledgements

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