Novel CE-MS Technique for Detection of High Explosives using Perfluorooctanoic Acid as a
MEKC and Mass Spectrometric Complexation Reagent

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Abstract

To address the need for the forensic analysis of high explosives, a novel capillary electrophoresis mass spectrometry (CE-MS) technique has been developed for high resolution, sensitivity, and mass accuracy detection of these compounds. The technique uses perfluorooctanoic acid (PFOA) as both a micellar electrokinetic chromatography (MEKC) reagent for separation of neutral explosives and as the complexation reagent for mass spectrometric detection of PFOA-explosive complexes in the negative ion mode. High explosives that formed complexes with PFOA included RDX, HMX, tetryl, and PETN. Somenitroaromatics were detected as molecular ions. Detection limits in the high parts per billion range and linear calibration responses over two orders of magnitude were obtained. For proof of concept, the technique was applied to the quantitative analysis of high explosives in sand samples.

Keywords: High explosives, CE-MS, MEKC, perfluorooctanoic acid complexation reagent, sand analysis

Introduction

High explosives constitute the majority of modern military and industrial explosive applications. [1] Moreover, high explosives such as TNT, RDX and PETN are commonly used in terrorist activities including the cargo bomb plot and attempts to blow up airplanes by explosives hidden in underwear. [2,3] In addition, because of their wide use, the environmental footprint of these compounds is an issue. [4, 5] Therefore, identification of high explosives and their post-blast degradation products is important both forensically due to their wide military use and by their potential acute and chronic effects on the environment and population. [6] Currently, GC-MS [7], HPLC-UV [8, 9], and HPLC-MS [10] are the preferred techniques for the analysis of explosives yet these techniques have some drawbacks.

Conventional GC-MS is the preferred technique for forensic analysis of explosives due to its availability, ease of use and low detection limits; however, it has some shortcomings for the analysis of high explosives such as RDX, HMX and tetryl. In the case of tetryl, one of its thermal degradation products, n-methylpicramide, is often detected. [10] GC-MS with a programmable temperature vaporizing (PTV) injector has been successfully used for the detection of high explosives including thermally labile compounds. [7, 11-14] High explosives are introduced into the injector at low temperature, minimizing their decomposition; subsequently the injector temperature is increased to introduce higher boiling point explosives into the column. On-column injection is yet another technique used for minimizing the decomposition of high explosives in GC-MS. [7] Additionally, to reduce the residence time of thermally labile high explosives in the GC oven, shorter columns can be utilized. [15] The PTV and on-column injection programs are part of a specialized inlet made to decrease thermal degradation of labile compounds. Most laboratories are not equipped with these types of injectors and are instead equipped with split/splitless inlets, as they are useful for most other GC-MS analyses. Therefore, thermally labile compounds such as HMX and RDX are difficult to detect using GC-MS systems present in most laboratories. Further, with the shorter column, HMX was still not detectable, and RDX produced a much lower signal by GC when compared to HPLC [15]. Moreover, GC-MS analysis of high explosive under electron impact ionization produces very weak molecular ions [12-13].

HPLC-MS is another analytical technique that has also been used for the analysis of explosives; however, because of the neutral nature of high explosives and thereby their low ionization efficiency under electrospray ionization (ESI), they are difficult to detect in both positive and negative ionization modes. To enhance the ionization of high explosives under HPLC-ESI/MS, a variety of additives have been incorporated into the mobile phase. Both pre-column and post-column addition of cationization and anionization reagents has been studied[16, 17]; however, cationization usually forms multiple components, which can complicate complex mixture analysis and reduce sensitivity. [18]Additives including ammonium formate or sodium nitrite have been used to promote complex formation to enable detection of the mono-nitrotoluenes (MNTs) and nitrobenzene.[19]

Capillary electrophoresis (CE) has also been utilized for the detection of explosives [20] but most explosives are neutral and are not separated by CE without the presence of a pseudo-stationary phase in the background electrolyte (BGE). To this end micellar electrokinetic chromatography (MEKC) is often employed [21-23]. Separation by MEKC is based on varied interaction of analytes with the micelles which result in separation if there are sufficient differences in hydrophobicity and electrophoretic mobility of analytes. [24]One commonly used MEKC reagent is sodium dodecyl sulfate (SDS); however, it causes ion suppression under electrospray ionization.[25]SDS was found to have poor affinity for HMX and RDX causing their elution to be close to the electroosmotic front.[26]Cyclodextrins (CD) have also been used as pseudo-stationary phases in CE to separate explosives. For example, nitroaromatic and cyclic nitramine compounds were analyzed by CE-MS using sulfobutylether- β -CD. [27] However, the authors reported complex CE-MS background spectra which made the identification of the explosive peaks very difficult. [27]To avoid the electrospray incompatibility and background issues of SDS and CDs, respectively, perfluorohexanoic to perfluorodecanoic have been introduced as volatile MEKC reagents. [24] It was found that perfluorooctanoic acid (PFOA) produced the best results for the analysis of pharmaceutical drugs and synthetic cannabinoids without impairing the MS signal over extended use.[24, 28]

In this manuscript we introduce an alternative method for the analysis of high explosives with high mass accuracy and minimal fragmentation which complements GC-MS analysis. The technique uses PFOA as an MEKC-MS reagent for the separation of and as a complexation reagent for high sensitivity electrospray ionization MS detection of high explosives and other

organic explosives. The purpose of this manuscript is to introduce a novel technology useful for the analysis of high explosives which would be applicable to a whole host of matrices of forensic interest including soil and water. The analysis in sand given as a real life example represents both a forensic and environmental application.

Materials and Methods

Analytical explosive standards EPA 8330 Mix A and Mix B (see Table 1 for list of compounds) and PFOA ammonium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). A standard of pentaerythritol tetranitrate (PETN) was purchased from Cerilliant Corporation (Round Rock, TX, USA). Contaminated sand (505989 and 506139) samples were provided by the US Army CBRNE Analytical & Remediation Activity Mobile Expeditionary Laboratory (CARA-MEL) (Edgewood, MD, USA). HPLC-grade acetonitrile (ACN) and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA), as well as ammonia and formic acid. Stock solutions of PFOA were made using deionized water. Nylon syringe filters with a 0.45 μm pore size were used to prepare the sample extracts for instrumental analysis.

Optimal BGE Selection

Optimization of the BGE was achieved using Beckman Coulter PA800 Plus (Fullerton, CA, USA) with UV/Vis diode array detection. A 90 cm (50 μm inner diameter) underivatized fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was conditioned with 1 M sodium hydroxide, deionized water, and BGE prior to each injection. Injections were performed at 0.5 psi for 10 seconds and separations were carried out using an applied voltage of +25 kV at 25°C. PFOA was selected as the surfactant for MEKC separation based on previous studies.[28] All aqueous solutions were prepared using deionized water. A stock solution of 200 mM PFOA was diluted to the desired PFOA concentration and buffered to a pH of 9.4 using 3 M ammonia. BGE solutions containing 15 mM, 25 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, and 100 mM PFOA were studied. Organic modifier addition to the BGE was also studied; however, no added benefit on the resolution or detection of the analytes was observed.

Sample Preparation

Individual explosive standards and EPA Method 8330 mixes A and B were prepared at varying concentrations in BGE for MEKC analysis. Sand samples labeled 506139 and 505989 were measured in ~5g portions and extracted with ACN according to EPA Method 8330 except no calcium chloride was added and they were sonicated for only 1 hour. The supernatant was removed from each sample and passed through a 0.45 μ m nylon syringe filter into a clean glass vial. The solvent was then evaporated completely and the residue was reconstituted in 50 μ L of ACN. After reconstitution, each extract was diluted with BGE for analysis.

Instrumentation MEKC-MS

MEKC-MS analyses were performed on a Beckman Coulter Proteomelab PA800 Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) interfaced with a high resolution Orbitrap Elite mass spectrometer (Thermo Scientific, Waltham, MA, USA) using a porous tip. [29] A 15 or 20 μ m inner diameter underivatized fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a total length of ~90 cm was used at 25°C. The capillary was conditioned for 6 minutes at a time using 1 M sodium hydroxide, followed by water, then methanol and finally BGE. Injections were made at 1 psi for 4 seconds and separations were performed using forward polarity mode (+25kV to inlet) and 5 psi of inlet pressure. Electrical connection was established by using 0.1% formic acid as the conductive liquid. [29] ESI-MS detection was performed in negative ion mode with a -1.1kV ESI voltage and the mass spectrometer heated capillary was held at 150°C to minimize PFOA-explosive complex fragmentation while maintaining efficient desolvation. Discussion of sensitivity refers to the S/N. Scan ranges included a low mass range of 100-350 m/z, intermediate mass range of 500-750 m/z, and high mass range 1000-2000 m/z, with a resolving power of 120,000.

Safety Precautions

When using EPA Method 8330, samples are meant to be homogenized however if explosive levels greater than 100 ppm are suspected, this should not be done due to possible explosion hazard. Further, some of the explosive compounds are sensitive to shock or heat so care should be taken to control the setting in which these standards are used.

Results and Discussion

Investigation of the interaction of PFOA with high explosives

Separation under MEKC is based on varied interaction of analytes with the PFOA micelles. To investigate if these PFOA-explosive complexes survive electrospray ionization/desolvation processes and can be detected by the mass spectrometer, several m/z ranges relevant to non-complexed and complexed compounds were scanned (Table 1). In the low mass range, m/z 100-350, deprotonated monoisotopic mass, $[M-H]^-$, of several explosives were observed including DNT, TNT and DNB. Moreover, HMX and RDX were seen as complexes with formate $[M+FA-H]^-$; while ADNT was seen as a complex with water $[M+H_2O-H]^-$. While no formate had been added to the sample or to the BGE; the formate adduct could have come from impurity in solvents as reported previously [26,30], or from the 0.1 % formic acid solution that was used as the conductive liquid around the porous tip of the capillary. At the intermediate m/z range (500-750), which encompassed PFOA-explosive complexes $[M+PFOA-H]^-$, HMX, RDX, PETN, tetryl, and ADNT were observed. Compared to uncomplexed forms, the PFOA-explosive complexes exhibited at least an order of magnitude higher sensitivity. To investigate if explosives and PFOA make higher aggregates, an m/z range of 1000-2000 was also studied for potential double complexations with the PFOA; however, no higher complexes were observed. Figure 1 displays the intermediate mass range, containing the $[M+PFOA-H]^-$ complex for HMX, RDX, PETN, tetryl and ADNT. Approximately, 0.14 nL containing 9.5 fmol of HMX, 12.6 fmol of RDX, 8.9 fmol of PETN, 9.8 fmol of tetryl, and 14.2 fmol of ADNT (2.8 pg of each compound) was injected. As shown in Figure 1, compared to the amounts injected, among high explosives, PFOA-HMX and PFOA-RDX complexes showed higher ionization efficiencies. The insets of Figure 1 show the mass spectra corresponding to electrophoretic peaks. At all PFOA concentrations studied, the two ADNT isomers were not resolved. The mono-nitrotoluenes and nitrobenzene were not detected under our experimental conditions.

Figure 1. CE-MS of High Explosives Standards. Extracted ion electropherograms of HMX, RDX, Tetryl, PETN and ADNT (from top to bottom) with insets showing the mass spectra across the peak.

Quantitative Studies for MEKC-MS

Quantitative studies were performed over a linear dynamic range of two orders of magnitude. MEKC-MS could detect signals from 200ppb to 20 ppm of the EPA 8330 Mix A and B and PETN. Figure 2 shows the calibration curves of the five explosives seen as complexes with PFOA. As shown, the R^2 values for all 5 complexes were >0.99 , indicating the viability of the complexes in quantitative analysis of high explosives.

Figure 2. Calibration curves of High Explosives Standards. Linear dynamic range for five high explosives demonstrating two orders of magnitude linear range.

Analysis of Environmental Samples

After successful separation and detection of explosives using PFOA as a MEKC reagent, several sand samples provided by the US Army CARA-MEL facility were tested using MEKC-MS. Figure 3, Panel A shows the extracted ion electropherograms of ADNT, HMX, and PETN found

in sand sample 505989 utilizing experimental conditions from Figure 1. Approximately, 0.14 nL of the sample was injected in the capillary. As shown in Figure 2, excellent $R^2(>0.99)$ were observed for these analytes, indicating the usefulness of the technique for the analysis of explosives in environmental samples. Using the calibration curves developed from the MEKC-MS method, the calculated concentrations of PETN and ADNT in the sand were 0.42 ppm and 0.14 ppm, respectively. Figure 3, Panel B shows the MEKC-MS analysis of sand sample 506139 which contained HMX, RDX and very low levels of tetryl and ADNT. This shows the utility of this technique for the analysis of high explosives which would be applicable to other matrices such as soil and water.

Figure 3. CE-MS of Sand Samples. Panel A: Extracted ion electropherograms of sand sample 505989 demonstrating detection of explosives at low-ppm to sub-ppm levels. Panel B: Extracted ion electropherograms of sand sample 506139.

Conclusions

MEKC-MS using PFOA as a MEKC reagent offers advantages when used for the separation, identification and quantification of explosives in environmental samples. In addition to facilitating the separation, PFOA forms complexes with high explosives, which exhibit high ionization efficiency under electrospray ionization. Moreover, the lack of significant background noise in the m/z range of PFOA-explosive complexes provides excellent detection limits for the explosives in EPA Method 8330 mixes A and B and PETN. Future work includes applications of this technique to identification and quantitation of explosives in other types of environmental samples with forensic relevance such as soil and water, as well as investigation of the use of other additives for the detection of MNTs and nitrobenzene. Overall, MEKC-MS could offer improved analysis to GC-MS and MEKC-UV if it were possible to detect the MNTs. This would allow for one analysis technique instead of having to use complementary analyses with multiple instruments.

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Table 1: List of compounds and their molecular information

Compound	Molecular Formula	Molecular Structure	Calculated Monoisotopic Mass [M]	[M-H] ⁻	[M+FA-H] ⁻	[M+PFOA-H] ⁻	[M+H ₂ O-H] ⁻
Pentaerythritoltetranitrate (PETN)	C ₅ H ₈ N ₄ O ₁₂		316.0139	315.0061	361.0116	728.9798	333.0167
EPA 8330 Mix A							
Nitrobenzene (NB)	C ₆ H ₅ NO ₂		123.0320	122.0242	168.0297	535.9979	140.0348
1, 3-Dinitrobenze (DNB)	C ₆ H ₄ N ₂ O ₄		168.0171	167.0093	213.0148	580.983	185.0199
2, 4-dinitrotoluene (2, 4-DNT)	C ₇ H ₆ N ₂ O ₄		182.0328	181.025	227.0305	594.9987	199.0356
2-amino-4, 6-dinitrotoluene (2-ADNT)	C ₇ H ₇ N ₃ O ₄		197.0437	196.0359	242.0414	610.0096	214.0465
1, 3, 5-Trinitrobenzene (TNB)	C ₆ H ₃ N ₃ O ₆		213.0022	211.9944	257.9999	625.9681	230.005
2, 4, 6-trinitrotoluene (TNT)	C ₇ H ₅ N ₃ O ₆		227.0178	226.0100	272.0155	639.9837	244.0206

Octahydro-1, 3, 5, 7-tetranitro-1, 3, 5, 7-tetrazocine (HMX)	$C_4H_8N_8O_8$		296.0465	295.0387	341.0442	709.0124	313.0493
1, 3, 5-trinitroperhydro-1, 3, 5-triazine (RDX)	$C_3H_6N_6O_6$		222.0349	221.0271	267.0326	635.0008	239.0377
EPA 8330 Mix B							
2-nitrotoluene (2-NT)	$C_7H_7NO_2$		137.0477	136.0399	182.0454	550.0136	154.0505
3-nitrotoluene (3-NT)	$C_7H_7NO_2$		137.0477	136.0399	182.0454	550.0136	154.0505
4-nitrotoluene (4-NT)	$C_7H_7NO_2$		137.0477	136.0399	182.0454	550.0136	154.0505
2, 6-dinitrotoluene (2, 6-DNT)	$C_7H_6N_2O_4$		182.0328	181.025	227.0305	594.9987	199.0356
4-amino-2, 6-dinitrotoluene (4-ADNT)	$C_7H_7N_3O_4$		197.0437	196.0359	242.0414	610.0096	214.0465
Tetryl	$C_7H_5N_5O_8$		287.0138	286.006	332.0115	699.9797	304.0166