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Cyclodextrin-assisted capillary electrophoresis for determination of the cyclic nitramine explosives RDX, HMX and CL-20 Comparison with high-performance liquid chromatography[☆]

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Abstract

A sulfobutyl ether–β-cyclodextrin-assisted electrokinetic chromatographic method was developed to rapidly resolve and detect the cyclic nitramine explosives 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaaza-isowurtzitane (CL-20), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and their related degradation intermediates in environmental samples. Development of the electrophoretic method required the measurement of the aqueous solubility of CL-20 which was determined to be 3.59 ± 0.74 mg/l at 25 °C (95% confidence interval, $n=3$). The performance of the method was then compared to results obtained from existing high-performance liquid chromatography methods including US Environmental Protection Agency method 8330.

Keywords: Explosives; RDX; HMX; CL-20; Nitramines

1. Introduction

The effort to produce lighter-mass munitions with greater explosive power has resulted in the addition of cyclic nitramines [e.g. 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaaza-isowurtzitane (CL-20), octa-hydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)] to supplement or replace aromatic energetic materials [*N*-2,4,6-tetranitro-*N*-methylaniline (Tetryl), 2,4,6-trinitrotoluene (TNT)] in rocket propellants and explosive formulations [1]. Structures for representative aromatic and cyclic nitramine explosives are shown in Fig. 1. With the exception of CL-20, which

is relatively new, the above compounds are identified as soil contaminants at explosive manufacturing or disposal sites and military training locations in Canada and the USA [2].

The environmental fate and toxicology of TNT and other aromatic explosives is fairly well established [3]. In contrast, the ecological fates of HMX and RDX and their degradation products are the subject of much current study [4,5], while almost no data are available for CL-20. Much information exists regarding the properties of these explosives as solid crystals, and defining their gaseous reaction products and energies of detonation. Controlled detonations of HMX and RDX exclusively produce low molecular mass products including nitrous oxide (N_2O), formaldehyde (HCHO), carbon dioxide (CO_2), and ammonia (NH_3) [6]. The detection of these compounds is a useful starting point for the identification of degradative pathways, and for fate
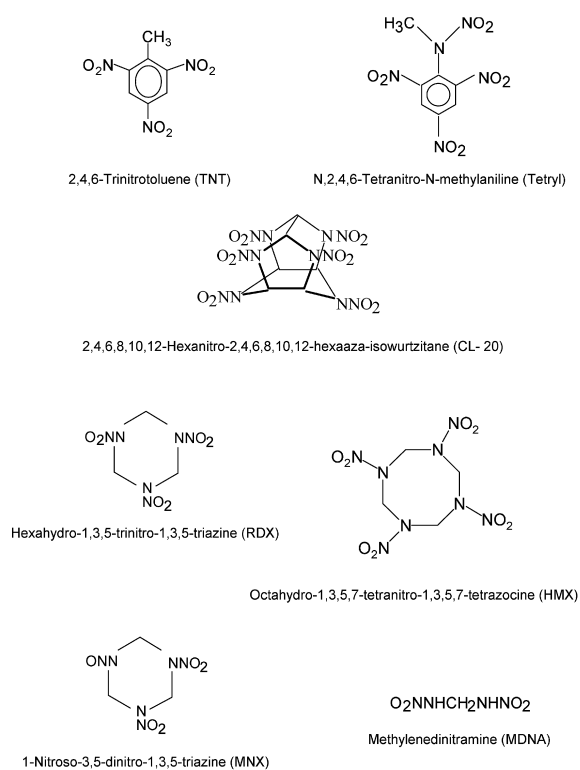

Fig. 1. Structural formulae of representative secondary aromatic and cyclic nitramine explosives.

and environmental impact assessment. To this end, much use is made of gas chromatography–mass spectrometry (GC–MS), solid-phase microextraction (SPME) and inorganic ion capillary electrophoresis (ICE) [7]. The degradation of explosives in soil and water, however, significantly differs from deflagration or detonation in at least four respects; (i) the reaction temperature is much lower, usually by 2–3 orders of magnitude, (ii) there is limited available reactant, usually in the mg/l range, (iii) the reactions occur in aqueous liquid environments, and (iv) relatively stable intermediates such as nitroso derivatives [8,9], methylenedinitramine [10] and 4-nitro-1,3-diaza-1-butanal [11] are observed. In the past, high-performance liquid chromatography, coupled to diode array UV detection (HPLC–UV) or electrospray mass spectrometric detection (LC–MS) have been used to analyze these explosives and their products in aqueous environmental samples [10]. Faster analytical methods are desirable, however, for the monitoring of chemical and enzymatic degra-

dations of this important family of energetic chemicals. Recently, sodium dodecylsulfate micellar electrokinetic chromatography (SDS-MEKC) [12] was employed in our laboratory for the rapid detection of TNT degradation intermediates, but this method does not adequately resolve nitramines which are much more polar in nature. Cyclodextrins may serve in the place of SDS micelles as pseudostationary ligands for the electrokinetic chromatographic (EKC) resolution of cyclic nitramines, as the polarity of the β-cyclodextrin inclusion cavity (approximately that of ethanol) is sufficiently matched with these analytes to allow complexation [13]. Charged cyclodextrins are routinely used for the resolution of enantiomeric pharmaceuticals [14], and have been applied for the CE-based analysis of aromatic explosives [15]. A sulfobutyl ether–β-cyclodextrin EKC method (CD-EKC) was developed for the detection of cyclic nitramines and some of their important degradation products in our laboratory.

2. Experimental

2.1. Materials

Epsilon CL-20 (purity 99.5%) was obtained from Thiokol Propulsion (Thiokol, UT, USA). RDX and HMX (with a purity >99%) were provided by Defense Research and Development Canada (DRDC) (Valcartier, Canada). Methylenedinitramine was obtained from Sigma–Aldrich (Oakville, Canada). Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) was obtained from SRI (Menlo Park, CA, USA). Advasep 4 sulfobutyl ether–β-cyclodextrin (tetrasodium salt, average degree of substitution 4), was purchased from CyDex (Overland Park, KS, USA). Tetryl, TNT, and all other nitroaromatic compounds were purchased from Supelco (Bellefonte, PA, USA). All other chemicals were obtained from Sigma as reagent grade.

2.2. Shaker flask determination of CL-20 aqueous solubility and stability

A 1 g/l stock solution of CL-20 in acetone was prepared and volumes ranging from 50 to 200 μl were added to 500-ml serum bottles with PTFE-

coated caps. Following the evaporation of acetone the bottles were filled with 500 ml deionized water. The bottles were agitated for 24 h in a Lab-line Environ Shaker at 37 °C. Ten-ml aliquots were then removed and allowed to equilibrate and settle at 25 °C for 4 h before analysis using HPLC–UV as described below.

2.3. HPLC–UV analysis

The RDX, HMX, and CL-20 concentrations were determined by HPLC using a Waters chromatographic system composed of a Model 600 pump, a Model 717 Plus injector, a temperature control module, and Model 996 photodiode array detector connected to a Dell GX200 computer running Millennium[32] (Waters) software. The column was a Supelcosil LC-CN (25 cm×4.6 mm, 5 μm; Supelco) maintained at 35 °C. The mobile phase (70% aqueous methanol) was run isocratically at 1 ml/min for the entire run time of 14 min. The detector was set to scan from 200 to 350 nm. Chromatograms were extracted at a wavelength of 230 nm with quantification taken from peak areas of external standards. Peaks were identified by comparison with elution times for external standards and by accompanying reference library diode array spectra as described earlier [10]. The injection volume was 50 μl. The limit of detection for this method was 0.02 mg/l (RSD=4.2%, $n=5$).

2.4. LC–MS analysis

The nitroso derivatives and ring cleavage products were analyzed by LC–MS with a Micromass Platform benchtop single quadrupole mass detector fronted by a Hewlett-Packard 1100 Series HPLC system equipped with a photodiode array detector. Samples (50 μl) were injected into a Supelcosil LC-CN column (25 cm×4.6 mm; 5 μm particle size; Supelco) thermostated at 35 °C. The solvent system consisted of a methanol–water gradient at a flow-rate of 1 ml/min. A first linear gradient was run from 10% to 20% methanol over 15 min followed by a second linear gradient from 20% to 60% over 5 min which was held for 3 min. This solvent ratio was returned to the initial conditions over 2 min and held for an extra 10 min. Analyte ionization was done in a negative electrospray ionization (ESI) mode produc-

ing mainly the deprotonated molecular mass ions $[M-H]^-$. The electrospray probe tip potential was set at 3.5 kV with a skimmer voltage of 30 V and an ion source temperature of 150 °C. The mass range was scanned from 40 to 400 u with a cycle time of 1.6 s and the resolution was set to 1 u (width at half-height). The detection limit was 0.004 mg/l (RSD=10%, $n=3$).

2.5. CE–UV analysis of nitramine explosives and their derivatives

MEKC separations with SDS were performed as described earlier [12]. Cyclodextrin-based EKC experiments (CD-EKC) were performed using a Hewlett-Packard (HP) [3D]CE instrument interfaced with a HP Vectra personal computer running HP Chemstation software. The HP [3D]CE system was fitted with a HP G-1600-31232 fused-silica bubble capillary with a total length of 64.5 cm, and an effective length (inlet to detection window) of 56 cm. The voltage was set at 30 kV and the temperature at 25 °C. Samples were injected by applying 50 mbar pressure to the capillary inlet for 5 s. The separation buffer was composed of 0.1 *M* sodium acetate buffer (pH 5.0) containing 10 m*M* sulfobutyl ether–β-cyclodextrin. Absorbances were monitored at wavelengths of 214, 230 and 280 nm. Unless otherwise indicated, the separation time was 18 min with 2 min separation buffer flushes of the capillary before each run. The total analysis time was therefore 20 min. The limit of detection for the method was 0.2 mg/l (RSD=5%, $n=20$).

3. Results and discussion

3.1. Polarity and aqueous solubility of RDX, HMX, and CL-20

CL-20 is a relatively new compound whose applications are currently under development, and many of its physicochemical properties are yet to be established. Epsilon CL-20, the most commonly used polymorph, is soluble in weakly polar organic solvents such as acetone (946 g/l) or ethyl acetate (450 g/l), but is insoluble in non-polar solvents such as toluene or benzene [16]. Its solubility in water was

not precisely determined and for this reason mea-surements were taken regarding CL-20 aqueous stability and solubility at ambient temperature. At 25 °C, the reported aqueous solubilities for RDX and HMX are 40.2 and 6.63 mg/l [2]. For CL-20, a maximum solubility of 3.59 ± 0.74 mg/l (95% confidence interval, $n=3$) at 25 °C (Fig. 2) was obtained from shake flask experiments. The hydrogen bonded lattice structure of the solvent (water) necessitates that solute polarity and molecular volume will have a strong effect on dissolution. The most common polymorphs of HMX, RDX, and CL-20 crystals have been examined using molecular dynamic simulations and X-ray diffraction to determine their molecular electronic structures and crystal packing densities [17–20]. For all three nitramines, polarized in-tramolecular electronic structures are observed, with partial charges of $-0.39e$ (e being the charge of a single electron) assigned to nitro group oxygens, and slight positive charges ($0.18e$) attributed to ring carbon nuclei. The three compounds are therefore weakly polar and dissolve readily in ketones such as acetone and to a lesser extent in alcohols such as methanol. They are not expected to dissolve exten-sively in non-polar solvents such as toluene, or in highly polar solvents such as *N*-methylformamide or water. The respective molecular volumes for RDX [17], HMX [18] and CL-20 [20] obtained from molecular dynamics estimates are 0.2118, 0.2607

and 0.3556 nl at 30 °C. The solubility of a solute in polar solvents decreases as molar volume increases, and this trend (i.e. solubility of RDX>HMX>CL-20) agrees with observed aqueous solubility mea-surements.

3.2. Resolution of cyclic nitramines using HPLC, SDS-MEKC and CD-EKC

The reversed-phase HPLC–UV method provides rapid, effective resolution for HMX, RDX and CL-20 (Fig. 3), with a quantitative detection limit of 0.02 mg/l. The use of 70% aqueous methanol mobile phase permitted the rapid detection of these analytes in 14 min, but under this condition methyl-enedinitramine was not retained by the column and for soil extracts the elution times of RDX nitroso-derivatives coincided with soil matrix peaks (data not shown). When the same liquid chromatographic system was used to front the electrospray ionization mass spectrometer, a gradient was applied which allowed for the observation of ring cleavage prod-ucts. The increase in resolution and sensitivity was compromised, however, by the longer run time which increased to 35 min per sample.

SDS-MEKC is an effective method for the rapid resolution of aromatic explosives. Fig. 4 is an SDS-MEKC electropherogram demonstrating the sepa-ration of 14 explosives identified as priority pollu-tants by the US Environmental Protection Agency
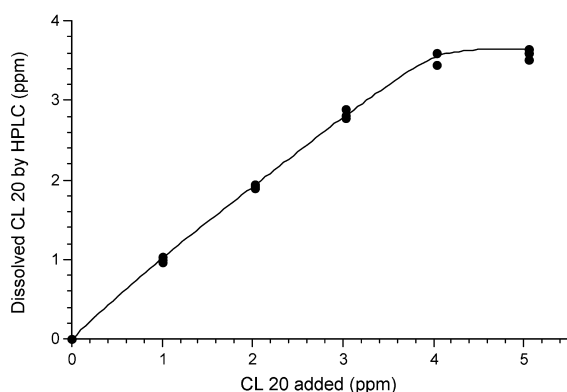

Fig. 2. Sub-saturation concentrations of 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaaza-isowurtzitane (CL-20) in water at 25 °C as detected by HPLC vs. CL-20 concentration as calculated by stock solution addition. The aqueous saturation concentration appeared to be 3.59 mg/l.
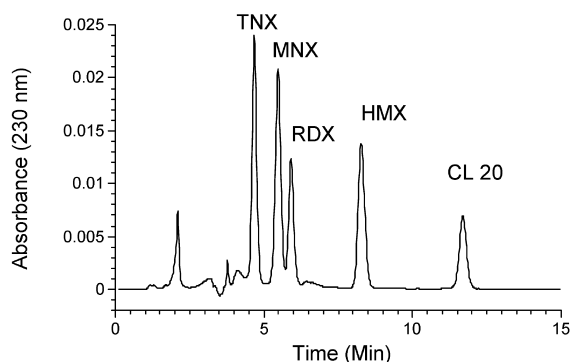

Fig. 3. Reverse phase HPLC–UV chromatogram of hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hexahydro-1-nitroso-3,5-di-nitro-1,3,5-triazine (MNX), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaaza-isowurtzitane (CL-20).
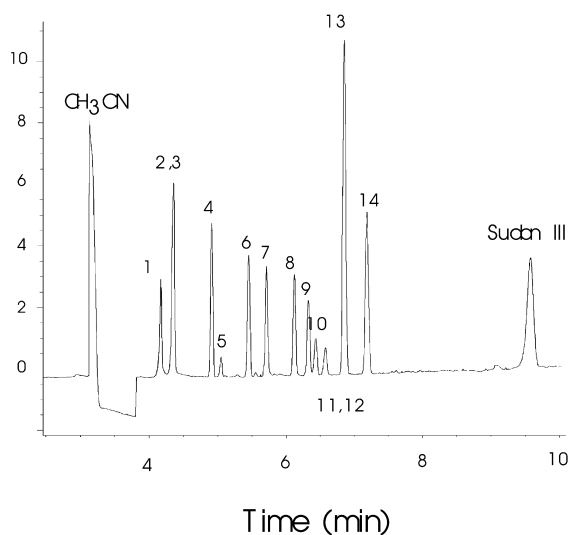
Fig. 4. MEKC separation of EPA method 8330 listed explosives in 12 mM sodium borate buffer pH 9.0, 50 mM sodium-dodecyl-sulfate, 25 °C. Peak identification: (1) octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), (2) hexahydro-1,3,5-trinitro-1,3,5-tri-azine (RDX), (3) 1,3,5-trinitrobenzene, (4) 1,3-dinitrobenzene, (5) nitrobenzene, (6) 2,4,6-trinitrotoluene (TNT), (7) N-2,4,6-tetrani-tro-N-methylaniline (Tetryl), (8) 2,4-dinitrotoluene, (9) 2,6-di-nitrotoluene, (10) 2-nitrotoluene, (11) 3-nitrotoluene, (12) 4-nitro-toluene, (13) 2-amino-4,6-dinitrotoluene, (14) 4-amino-2,6-dinit-rotoluene.

(EPA) method 8330 [21]. RDX and HMX partition poorly with SDS micelles and are observed to migrate close to the electroosmotic front. The polarity of the β-cyclodextrin cavity is estimated to be close to that of ethanol [13] and would therefore be more suitable for the solubilization of RDX, HMX, CL-20, and their degradation products. For cyclo-dextrins, however, the electrokinetic resolution of uncharged species is not solely based on polarity and hydrophobic interaction, but also on the geometry of the host–guest complexes. As previously stated, the molecular volumes for RDX [17], HMX [18] and CL-20 [20] are 0.2118, 0.2607 and 0.3556 nl at 30 °C. The volume for the β-cyclodextrin cavity is ~0.346 nl [13,22]; a value which allows for the complete inclusion of HMX and RDX, but restricts the complexation of CL-20. As indicated in Fig. 5, the three nitramines were observed to form complex-es with sulfobutyl ether–β-cyclodextrin, and resolve electrophoretically.

Faster separations are possible using higher-pH

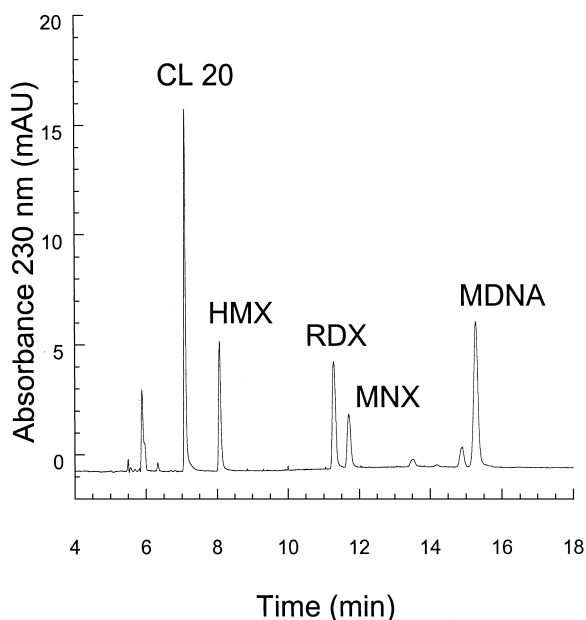Fig. 5. Sulfobutyl ether–β-cyclodextrin-based CE separation of cyclic nitramines in 0.1 M sodium acetate, pH 5.0, containing 10 mM SB–β-cyclodextrin.

separation buffer, but cyclic nitramines and their degradation products are known to be stable in acid solution [6] and for this reason sodium acetate/acetic acid buffer (pH 5) was employed. The degradation products mononitroso-RDX (MNX) and methyl-enedinitramine (MDNA) also form complexes with sulfobutyl ether–β-cyclodextrin and their peaks are identified in Fig. 5. These compounds have been observed frequently in RDX degradation experiments using commercially obtained nitrate reductase en-zyme [23], and domestic anaerobic sludge [10]. At present, the CD-EKC method is better applied to the rapid detection of cyclic nitramines and ring cleav-age products in real time, while the HPLC (MS) method offers a 50-fold lower limit of detection in comparison with the CD-EKC or SDS-MEKC meth-ods [0.004 mg/l (RSD=10%, $n=3$) vs. 0.2 mg/l (RSD=5%, $n=20$) and 0.5 mg/l (RSD=4%, $n=5$), respectively].

4. Conclusions

The solubility of the cyclic nitramine explosives

RDX, HMX, and CL-20 (40, 6.6 and 3.6 mg/l, respectively) in aqueous solution was observed to depend on analyte polarity and molecular volume. A comparison of reversed-phase HPLC, SDS-MEKC, and sulfobutyl ether–β-cyclodextrin EKC for the resolution of these compounds and their degradation products favoured the use of HPLC and CD-EKC. The HPLC method offers a lower detection limit (0.004 mg/l, RSD=10%, n=3), while the faster CD-EKC method facilitates real-time monitoring of experiments. The HPLC and the CD-EKC methods can be considered complimentary to each other in the determination of cyclic nitramines and their degradation products in environmental samples.

Acknowledgements

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