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Determination of Nitrate Carry-Over on Bytac® Strips Via Capillary Electrophoresis

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14. ABSTRACT

Inorganic explosives including ammonium nitrate, urea nitrate, and ANFO are among the most common energetic materials used in home-made devices. The ability to detect these materials sensitively with low occurrences of false negatives and positives is of paramount importance. As new detection technologies are developed, the need to standardize procedures to evaluate these technologies is equally important. Most technologies include a sampling step; typically a substrate used to swipe a suspect surface. A key performance metric is the transfer efficiency of this sampling step. Capillary electrophoresis was used to quantify the nitrate ion on cardboard-back Teflon strip (Bytac®) to establish the transfer efficiency for three inorganic explosives. These results indicate that transfer efficiency is analyte specific, dependent upon the amount of analyte present, and the mechanism of transfer.

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Introduction

Technologies including IMS and colorimetric detection kits are currently being deployed throughout the world in order to detect explosives materials. The first step in applying these technologies is the act of transferring the explosives material from a suspect surface and transporting it to the analytical instrument. While it is important to establish limits of detection for these techniques, it is equally important to understand the efficacy of transfer as it relates to substance, concentration, and sampling technique.

Capillary electrophoresis (CE) has been used for the analysis of various inorganic salts with an eye towards explosives detection[1,2]. Those efforts primarily focused on the analysis of complex samples and endeavored to provide a comprehensive picture of the cation and anion signature of post-blast residues. While nitrate is the primary anion of interest in many improvised explosives devices, simultaneous detection of sulfate, chlorate, chloride, ammonium, urea, and aluminum allows for a more precise picture of the explosives material used.

NRL was recently tasked with evaluating a device that has the potential of being utilized in real world environments for the detection of inorganic explosives. During the course of this evaluation, it became obvious that no routine method for evaluating transfer efficiency has been established in the literature for the analytes of interest in this test: specifically, ammonium nitrate (AN), urea nitrate (UN), and ANFO. Given the relative lack of complexity of these samples, we determined that qualitative and quantitative analysis for nitrate via capillary electrophoresis was a viable option.

This report summarizes the development of a CE-based method for the analysis of nitrate from Bytac® strips. Transfer efficiency as a function of analyte, concentration, and swipe method are presented and discussed.

Experimental Method

The overall separation method is modified from reference 2 and has been previously implemented at NRL for the analysis of ozone monitoring badges (NRL Ltr Report 3905 Ser 6180/0345; September 28, 2006).

Instrumentation

All separations were performed on a Beckman Coulter P/ACE MDQ capillary electrophoresis instrument. A 60 cm long (10 cm effective length), 75 µm i.d. bare fused-silica capillary was used in all of the separations presented.

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[1] J.P. Hutchinson, C.J. Evenhuis, C. Johns, A.A. Kazarian, M.C. Breadmore, M. Macka, E.F. Hilder, R.M. Guilt, G.W. Dicinoski, P.R. Haddad, *Analytical Chemistry*, **2007**, *79*, 7005-7013

[2] F. Tagliaro, F. Bortolotti, G. Manetto, V.L. Pascali, M.Marigo, *Electrophoresis*, **2002**, *23*, 278-282.

Manuscript approved September 22, 2011.

Injection and Separation Scheme

The background electrolyte (BGE) for the separation was 100 mM sodium tetraborate (pH 8.9). The sample matrix was 5 mM sodium tetraborate and included 50 µM potassium bromide as an internal standard. Prior to analysis, the capillary was conditioned with 1 M NaOH for 1 minute at 30 psi, water for 1 minute at 30 psi, then with BGE for 2 minutes at 30 psi. Sample was injected hydrodynamically for 23 seconds at 1 psi, generating a 5 cm sample plug. The sample plug was pushed into the capillary another 0.5 cm in order to ensure that no analyte escaped into the inlet BGE vial during the initial moments of the separation.

Due to the high concentration of the BGE, electroosmotic flow (EOF) is significantly reduced allowing for the application of a negative potential in order to selectively detect the anions in the sample matrix. Specifically, a potential of 10 kV (reversed polarity) was applied across the capillary. The separation was complete in less than 5 minutes, with a total analysis time including capillary flushing/injection/separation occurring in 10 minutes.

Sample Preparation

Ionic explosives of interest were prepared in ethanol and deposited onto 1 inch square Bytac® strips. Specifically, stock solutions of AN, UN, and ANFO were prepared at 6.4, 64, and 640 ng/µL; 100 µL of a given solution was deposited in a grid-like pattern on the strip as 10 µL subunits (droplets). The solvent was allowed to evaporate to dryness. After drying, for the highest concentrations, an obvious crystalline structure was observed at the location where drops were deposited. These analytes are known to be hydroscopic; therefore, the prepared strips were stored in a chamber containing Drierite in order to limit water contamination on the strip.

Sample Transfer Technique

The transfer substrate (the material to which explosives were to be transferred to/from the Bytac® strip) was placed face-up on a stainless steel platform. The explosives contaminated Bytac® strip was placed face-down upon the transfer substrate. The substrate to which transfer was to occur was placed upon the Bytac® strip. A homemade press providing 10.4 pounds per square inch of pressure was used to facilitate transfer from the Bytac® to the transfer substrate for 15 seconds. After this time, the press was disengaged and the sampling substrate separated from the Bytac®. The Bytac® strip was analyzed for any retained inorganic explosive materials. Alternatively, for a few experiments, the transfer substrate was placed flat upon a surface and the Bytac® strip was swiped over the surface in a single motion.

Sampling from Bytac® Strips

The inorganic explosives were sampled from the Bytac® strip via extraction into the sample matrix described in the *Injection and Separation Scheme* section. Briefly, 50 µL of sample matrix (5 mM sodium tetraborate, 50 µM potassium bromide) was taken into a pipette.

This solution was pipetted back and forth over the surface of the Bytac®, with special attention taken to ensure that the liquid dissolved any obvious crystals from the surface. Typically, 15-20 back-and-forth pipetting events occurred. The solution was stored in a 250 µL sample vial until analysis via CE. The small sample volume assured detection of nitrate at very low levels.

Results and Discussion

Nitrate Separation

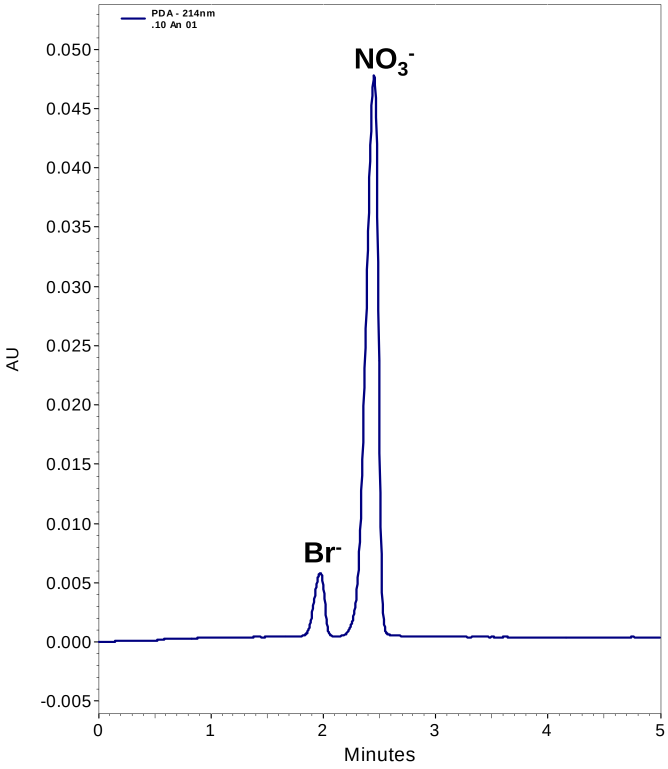

Figure 1. Electropherogram of an ammonium nitrate sample. Sample was 0.64 µg of AN extracted into 50 µL of sample matrix as described in the experimental section.

A representative electropherogram of the nitrate separation is presented in Figure 1. The first peak is the internal standard, bromide, while the second peak is nitrate. The ammonium ion and the potassium ion (counter ion to bromide) are not seen in the electropherogram due to the reversed polarity; electroosmotic flow suppression is sufficient to cause the ammonium and potassium to migrate out of the capillary into the inlet side BGE vial during separation.

A calibration curve was prepared for nitrate concentrations from 6.2 to 0.04 ng/µL. The results indicate a linear relationship between the normalized peak area of nitrate and concentration (R^2 = 0.9992). This indicates that quantitation is possible, however in this work we chose only to compare control experiments (known amount of sample deposited onto Bytac®) to post-transfer samples.

Background Nitrate Levels

One of the key difficulties in nitrate analysis is that nitrates are so abundant in nature. With little difficulty, trace contamination of nitrates is possible simply by excessive handling. In fact we noted that after several days of routine use, we detected trace levels of nitrate in the sample matrix solution. This was likely due to nitrate transfer from pipette tips; that is not to say that pipette tips were reused, simply that as supplied by the manufacturer, there is some nitrate contamination. This contamination is so low that it did not affect our results in any way.

Given the pervasiveness of nitrates, two sets of blanks (no exposure to any of the explosives) were prepared. The first blank involved simply sampling off of a fresh Bytac® strip as described in the experimental section. In that instance an average normalized peak area for nitrate was observed of 1900 units – or approximately 2 ng of nitrate. The second was a method blank, where the transfer substrate was used as indicated in the experimental section. These result indicated that there was some transfer of nitrate from the transfer substrate to the Bytac® strip with approximately 10 ng of nitrate detected.

Inorganic Explosives Sampling

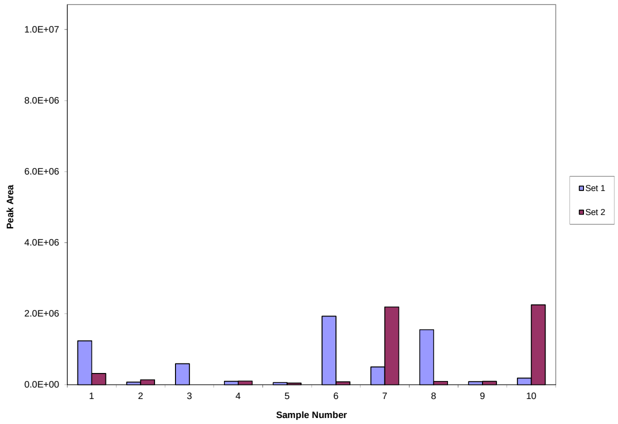

Figure 2. Remaining nitrate on Bytac® strips with 64 µg of ammonium nitrated added.

Initial experiments were performed on Bytac® strips that had 64 µg of ammonium nitrate deposited onto the surface. A total of 18 control strips were prepared to establish the signal associated with the control (no transfer). The relative standard deviation for the extraction technique was 10% - a very reasonable number given the simplicity of sampling. A total of 20 strips were used in transfer experiments in two sets of 10. The raw data are presented in Figure 2. Full scale of the graph represents average peak area of the control experiment. The column height in the graph is indicative of the amount of nitrate remaining on the strip. The relative standard deviation of the amount of remaining nitrate is very high at 129%, indicating that the transfer efficiency is highly variable. That being said, the overall transfer efficiency is 94%, indicating that the press method outlined in the experimental section is viable for the transfer of ammonium nitrate at a high concentration.

In a similar fashion, experiments were performed on strips prepared with 6.4 µg of ammonium nitrate and are presented in Figure 3. As in the previous experiment, full scale on the figure represented the amount of nitrate detected on the controls. In this instance, two sets of 10 were sampled using the press method (Set 1 and Set 2) and 2 sets of 10 were sampled using the swipe method (Set 3 and Set 4). The relative standard deviation for transfer efficiency for the press method samples is 43% with a transfer efficiency of 82%. For the swiped samples, the relative standard deviation is reduced to 14% and the transfer efficiency improves to 88%.

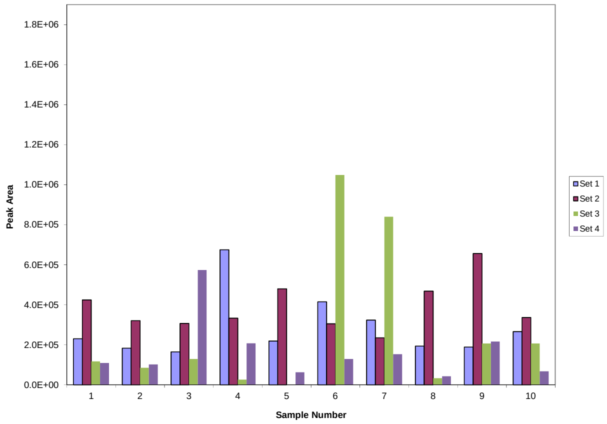

Figure 3. Remaining nitrate on Bytac® strip with 6.4 µg of ammonium nitrate added.

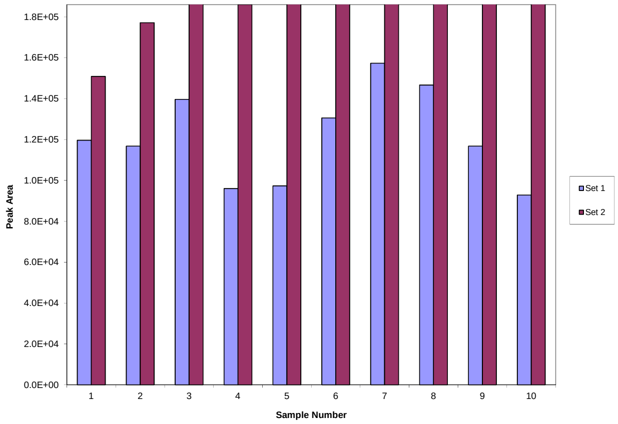

Figure 4. Remaining nitrate on Bytac® strip with 0.64 mg of ammonium nitrate added.

A final experiment was performed on strips prepared with 0.64 µg of ammonium nitrate (Figure 4). In this instance, the transfer efficiency is very poor, with nearly the entire second set showing no transfer of ammonium nitrate from the Bytac® strip. The source of variability from Set 1 to Set 2 is unknown at this time, but it should be noted that the efficiencies are statistically different. Both sets of samples were prepared at the same time, several hours prior to analysis. The relative humidity of the room where the samples were stored and the room where sample transfer occurred were not significantly different. In fact the only obvious difference between the samples was that Set 2 was analyzed approximately 45 minutes after Set 1. Ammonium nitrate is hygroscopic, so it is possible that the time a sample is allowed to sit around may result in transfer efficiency differences, however, no further experiments were performed to address that concern.

Inorganic explosives UN and ANFO were also analyzed. All results are summarized in Table 1. For urea nitrate, there was a large disparity between press-based transfer and swipe-based transfer. Press results indicate that no transfer occurs from the Bytac® strip to the transfer substrate for the 64 µg samples, whereas 93% transfer efficiency is achieved using the swipe technique. While the transfer efficiency is high, it should be noted that the reproducibility of the swipe technique is poor, with a relative standard deviation of 179%. Improved transfer efficiency is observed using the press technique for 6.4 µg samples; however it is still very poor with only 11% efficiency. The swipe technique was very effective on the 6.4 µg sample of UN with 90% transfer efficiency. Transfer efficiency for the 0.64 µg samples was 26%. These results are dissimilar to the ammonium nitrate sample, potentially indicating analyte specific behavior. ANFO results are generally consistent with those obtained for AN, which is not surprising given that ANFO is simply AN with the addition of diesel fuel (approximately 5% by weight). Transfer efficiencies exceeded 80% for all ANFO sampled regardless of sampling technique, save for the 0.64 µg sample which is 26% efficient.

Table 1 . Summary of test results

Explosive Sample	*Sample Technique*	*Transfer Efficiency (%)*	*Rel. Standard Devation (%)*	*Number of Samples*
64 μg AN	Press	94	129	19 samples, 18 controls
6.4 μg AN	Press	82	43	20 samples, 13 controls
6.4 μg AN	Swipe	88	15	19 samples, 13 controls
0.64 μg AN	Press	9	34	20 samples, 20 controls
64 μg UN	Press	-3	12	20 samples, 19 controls
64 μg UN	Swipe	93	179	20 samples, 19 controls
6.4 μg UN	Press	11	12	20 samples, 20 controls
6.4 μg UN	Swipe	90	51	9 samples, 20 controls
0.64 μg UN	Press	27	21	20 samples, 20 controls
64 μg ANFO	Press	87	142	19 samples, 19 controls
64 μg ANFO	Swipe	98	219	20 samples, 19 controls
6.4 μg ANFO	Press	81	44	20 samples, 6 controls
6.4 μg ANFO	Swipe	94	109	10 samples, 6 controls
0.64 μg ANFO	Press	26	25	20 samples, 20 controls

Conclusions

This work demonstrates that capillary electrophoretic analysis of inorganic explosives is a viable method for the determination of transfer efficiency from Bytac® strips. Using a pipette to extract remaining salt from the strip into an appropriate sample matrix is reproducible with only a 10% relative standard deviation. The results indicate a transfer efficiency dependence on both sample type and mass present. In total, 470 individual samples were prepared and analyzed within a work week, indicating that this technique can offer the end user a rapid and effective analytical tool for the evaluation of emerging explosives detection technologies.

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