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Improved Separation of Organic Explosives by Modified Micellar Electrokinetic Capillary Chromatography

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Modified Micellar Electrokinetic Capillary Chromatography에 의한 폭약 성분의 분리능 향상

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Abstract : Among various CE separation methods, micellar electrokinetic capillary chromatography(MECC) method using sodium dodecylsulfate(SDS) provides rapid and accurate separation of organic explosive constituents with easy. The running buffer was composed with 2.5 mM borate and 25mM SDS(pH 8.5). Addition of 1M urea and 10% organic modifiers (acetonitrile, methanol and ethanol) improves the resolution of adjacent explosive constituents. When 15 explosive constituents were developed in MECC, most constituents were separated successively while RDX/TNB and DNN/DEP were not, and detection limits of separated compounds are in range of 1 to 4 ppm.

요약 : 계면활성제로 sodium dodecylsulfate(SDS)를 이용한 micellar electrokinetic capillary chromatography(MECC) 방법을 이용하여 유기 폭약 성분을 신속하고 정확하게 분석할 수 있는 방법을 연구하였다. 완충용액으로는 2.5mM borate와 25mM SDS(pH 8.5)를 사용하였고, 완충용액에 1M urea와 10% 유기 용매(acetonitrile, methanol 및 ethanol)를 첨가한 결과 분리능이 향상되는 현상을 관찰할 수 있다. 15종의 폭약 혹은 그 구성 성분을 MECC 방법으로 분리한 결과 RDX/TNB 및 DNN/DEP를 제외한 성분을 분리할 수 있었고, 검출한계는 1~4ppm으로 나타났다.

Key words : micellar electrokinetic capillary chromatography, explosive constituents, sodium dodecylsulfate, urea, organic modifier

1. Introduction

Separation and identification of explosives or their burned flake residues are one of the most important task in forensic investigation. Forensic chemists analyse bomb fragments or residues to

determine the type of explosive materials used. Modern explosives are mainly composed with explosive inorganic or organic materials, oxidizer, sensitizer, plasticizer, stabilizer and fuels.[1] Over the years, various methods have been developed for the analysis of the inorganic constituents of gunshot residue(GSR) and unburned explosives flakes. For the analysis of metals such as antimony, barium and lead, sophisticated and expensive techniques are often employed. As for the organic materials, the ability to detect anions such as nitrate, nitrite, chloride and so on, is imperative for it provides the key in revealing the nature and source of the explosive materials. For this purpose, neutron activation analysis(NAA), atomic absorption spectroscopy(AAS), scanning electron microscopy-energy dispersive X-ray analysis(SEM-EDXA) which give sufficient sensitivity and specificity to those elements provided satisfactory results. Also, ion chromatography(IC) has been suggested as the most powerful tools for analysis of anions present in explosive materials and their residues.[5~7] However, routine application has been limited due to interferences, high blank results, prohibitive instrument cost(especially SEM-EDXA and NAA) and analysis time.[2,3] Furthermore, the results obtained with those analytical techniques are not always considered as conclusive legal proof in judicial proceedings because of possible environmental or occupational contamination.[4]

Since commercial ammunition and explosives devices contain a mixture of explosives, stabilizers and plasticizers, analysis of these characteristic organic constituents, listed in *Table* 1, for forensic purpose has gained recent attentions. Thin-layer chromatography(TLC) had been proposed as a quick and inexpensive technique for the detection of principal organic compounds of propellant discharge.[8,9] This method, however, has less sensitivity than gas chromatography coupled with mass spectroscopy(GC/MS)[10] or high performance liquid chromatography(HPLC).[11,12] Thermally stable organic constituents of explosive materials are commonly analyzed by GC/MS, whereas HPLC are used for the more thermally labile constituents.

Separation by CE provides a number of advantages such as high resolution that may achieved on short analysis time and microsample amount with an automated matter.[13] MECC is a hybrid of CE in which a charged micellar agent is added to a

Table 1. Organic explosives and additives

Species	Compounds	Abbreviation
Nitroaromatics	2,4-dinitrotoluene	2,4-DNT
	2,6-dinitrotoluene	2,6-DNT
	3,4-dinitortoluene	3,4-DNT
	trinitrotoluene	TNT
	trinitrophenol(picric acid)	PA
	dinitrobenzene	DNB
	trinitrobenzene	TNB
	dinitronaphthalene-mixutre	DNN
Nitramines	1,3,5-trinitro-1,3,5-triazacyclohexane	RDX
	2,4,6-N-tetranitro-N-methylaniline	Tetryl
Nitric ester	nitroguanidine	Ng
Phthalates	dibutylphthalate	DBP
	diethylphthalate	DEP
Amines	diphenylamine	DPA
	2-nitrodiphenylamine	2-NDPA

electrolyte to separate neutral compounds.[14~16] Selective partitioning of the analyte into the micellar phase causes them to migrate at different flow rates from that of the bulk electroosmotic flow rate. Work by Northrop et al.[15] and Kleibohmer et al.[16] demonstrated the use of MECC with SDS as a surfactant and γ-cyclodextrine as a selectivity enhancing additives for organic GSR and explosives. Hargadon and McCord also had reported the analysis of anions in explosive residue by CE.[7] The separation of ionic solutes in MECC was improved by adding a tetraalkylammonium salt to the buffer by mediating interactions of the analytes with the micelles.[8]

In this work, the separation method of MECC for the organic constituents found in explosive materials was modified based on the work of Northrop et al.[15] and Kleibohmer et al.[16] The effect on electroosmotic velocity to electropherogram by an addition of urea and/or organic solvent to the buffer were evaluated.

2. Experimental

2.1. Apparatus

The CE system used for the study was a ABI 270A-HT equipped with a 0~30kV power supply, and a variable UV/Vis detector(190~700nm). It has two automatic injection modes of hydrostatic and electromigration. A 63cm of uncoated polyimide fused silica capillary colum having 50㎛ inner diameter was used in this CE system. The effective column length(from start to detector) was 50cm.

2.2. Reagents

SDS and sodium tetraborate and Sudan Ⅲ were purchased from Sigma Chemical Co. (St. Louis, MO). All explosives including TNT, Tetryl and RDX were obatined by a courtesy of the Han Wha Co. (Seoul, Korea). The other explosive additives listed in *Table* 1 were purchased from Tokyo Kasei Co. (Tokyo, Japan)

The electrolyte used in the separation contained 2.5mM of borate buffer at pH 8.5 and 25mM of SDS. Urea were tested as selective enhancing additive at various concentrations. The effect of organic solvents(acetonitrile, methanol and ethanol) were tested with 10% solution buffer. The buffer solutions were filtered through a membrane filter with 0.45㎛ pore size.

2.3. Procedures

Standards : The 1% explosive standards and 0.1% nitroguanidine stock solutions were prepared in acetonitrile. The standard working solutions were made by diluting stock solution into appropriate concentration with electrolyte buffer.

Electrophoretic condition : Hydrostatic injection was employed for 1.5 seconds. The separation was carried out with a 20kV running voltage. Detection was performed under UV at 250nm.

3. Results and Discussion

3.1. Analysis of standard samples

Stock solutions were made up by dissolving explosives and additives in acetonitrile, listed in *Table* 1 except PETN, NG and NC. PETN and NG were excluded in this experiment due to the fact that the purchased PETN concentration was not labeled. As for the NG, we were not satisfied with the purity of NG and needed to exclude it from our experiment. We also excluded the NC because it had a poor solubility in acetonitrile. The standard solutions were made by diluting each stock solution to 10ppm with buffer. The standard mixture of the 15 organic constituents were eluted into CE system with a buffer containing 2.5mM borate and 25mM SDS(pH 8.5) according to the previous report[15] and yielded the electropherogram as shown in *Fig.* 1. A 10ppm internal standard of benzoic acid(BA)

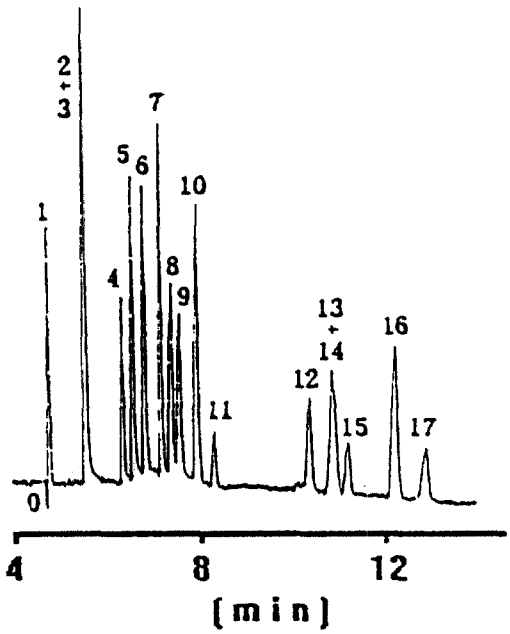

Fig. 1. Separation of explosives constituents at 250 nm without urea and organic solvents
0 : acetonitrile, 1 : Ng, 2 : RDX, 3 : TNB, 4 : DNB, 5 : TNT, 6 : Tetryl, 7 : 2,4-DNT, 8 : 2,6-DNT, 9 : 3,4-DNT, 10 : picric acid, 11 : Benzoic acid(INSTD), 12 · 13 : DNN-mix, 14 : DEP, 15 : DPA, 16 : 2-NDPA, 17 : DBP

was integrated into the standard mixture. As expected, acetonitrile peak was eluted first(negative peak), followed by the additives. However, in that separation condition, baseline resolution was not achieved. RDX was overlapped with TNB, and one of DNN isomer's peak was overlapped with DEP. Sudan Ⅲ which was used as an indicator for micelle migration follows DBP. Sudan Ⅲ which was used on as an indicator for migration follows DBP. The obtained electorpherogram certainly required better separation efficiencies in order to decuce the source of the explosives accurately.

3.2. Calibration curves and detection limits

The linearity of calibration curves and detection limits achieved by the present method are shown in *Table* 2. Because of high resolution of the MECC method excellent linearities to most of organic constituents in the explosive were obatained. Approximate detection limits(S/N=3) were also deternimed from concentration analysis and the range was found to be between 0.5~4ppm (around 5×10^{-6} mol/L). In previous reports[15,16], detection limits were approximately estimated as under 5×10^{-6}mol/L for aromatic compunds and 1×10^{-5}mol/L for aliphatic compounds. Since each component has individual UV absorption characterisitics, the analysis at different wavelengths for each components can provide better sensitivity and detection limits.

3.3. Addition of urea

Table 2. Regression equations and detection limits of explosive constituents

Compounds	Regression equations			Detection
	Slope	Y intercept	r squared	Limits(ppm)
Ng	0.153	−0.041	0.978	1.0
RDX	0.203	−0.219	0.981	0.9
DNB	0.220	−0.319	0.998	0.9
TNT	0.351	−0.916	0.945	0.6
Tetryl	0.351	−0.634	0.989	0.7
2,4-DNT	0.364	−0.990	0.942	0.5
2,6-DNT	0.221	−0.418	0.988	1.1
DEP	0.094	−0.201	0.991	2.5
DPA	0.088	−0.203	0.987	3.0
2-NDPA	0.222	−0.641	0.940	1.1
DBP	0.052	−0.257	0.930	4.0

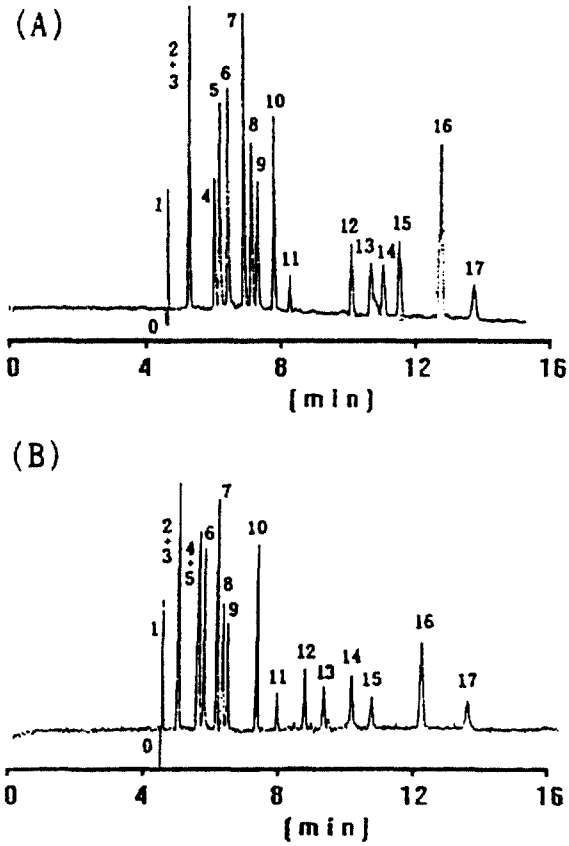

Fig. 2. The comparison of migration with 1M urea(A) and 3M urea(B) addition to the running buffer

Urea was usually added to running buffer in order to enhance the solubilities of viscous or near insoluble materials. Urea was also added to decrease the electroosmotic velocity in CE.[16] In looking for ways to improve resolution of the overlapped explosives' peaks, we decided to add urea into the SDS buffer. The concentration effect of urea was examined using four different concentrations of 0, 1, 3 and 6M, and the results were shown in *Fig.* 2, As shown in *Fig.* 2. the best result was obtained by addition of 1M urea. We did not include the electrophoregram obtained with 6M urea in *Fig.* 2, even though a high concentration usually have been used in CE experiments.[17] The addition of 1M urea leads to a separation of DNN and DEP peaks, but not for RDX and TNB. Overall resolutions of explosive additives were marginally improved in comparison to those results obtained without the addition of urea. With a higher concentration than 1M of urea, the resolution for certain additives were increased whereas the resolution of the other compounds were decreased.

The effects of concentration on capacity factor (k′) value are listed in *Table* 3. The k′ value between DNB and TNT decreased as the concentration of urea increased (0.071 for 1M and 0 for 3M urea), even co-eluted both compunds at over 3M of urea. On the other hand, the difference between DNN and DEP was increased from 0.931 at 1M to 1.583 at 3M urea. These phenomena are demonstrative of a change in distribution ratio for the analyte to micelle in buffer and in electroosmotic velocity. Actually, k′ values were decreased with increasing concentration of urea. This effect became more obvious for nitro compounds. Even though k′ values were decreased with the increase of urea concentration, the analysis time was increased due to a decreased electroosmotic velocity.

3.4. Organic solvents effect

The effects on the k′ values from the addition of various miscible organic solvents to buffers were examined. Three different solvents (acetonitrile, methanol and ethanol) were added into the buffers, respectively, by adjusting their V/V concentrations to 10%. *Fig.* 3 shows the effect of various organic solvents on their electropherograms. The electorpherogram of trace A in *Fig.* 3 were obtained with a 10% acetonitrile buffer. Under this condition, RDX and TNB could be resolved, but the resolution of Tetryl and 2, 4 DNT was decreased to zero, and a reversed migration order of DNN and DEP was observed. The result with 10% methanol in buffer shows similar effect as that with acetonitrile(*Fig.* 3. B). However, as shown in *Fig.* 3 trace C, the electrophoregram with 10% ethanol in

Table 3. The comparsion of capacity factors with various concentration of urea

Compounds	Capacity factors(K′)			Compounds	Capacity factors(K′)		
	No urea	1M urea	3M urea		No urea	1M urea	3M urea
Ng	0.027	0.017	0.026	3,4-DNT	1.530	1.209	0.862
RDX+TNB	0.326	0.226	0.205	Picric acid	1.838	1.525	1.421
DNB	0.706	0.545	0.451	DNN-1	5.902	4.187	2.705
TNT	0.834	0.616	0.451	DNN-2	7.765	5.403	3.445
Tetryl	0.987	0.729	0.521	DEP	7.765	6.334	5.028
2,4-DNT	1.223	0.962	0.710	DPA	9.109	8.136	6.655
2,6-DNT	1.390	1.097	0.792	2-NDPA	22.392	18.246	16.893

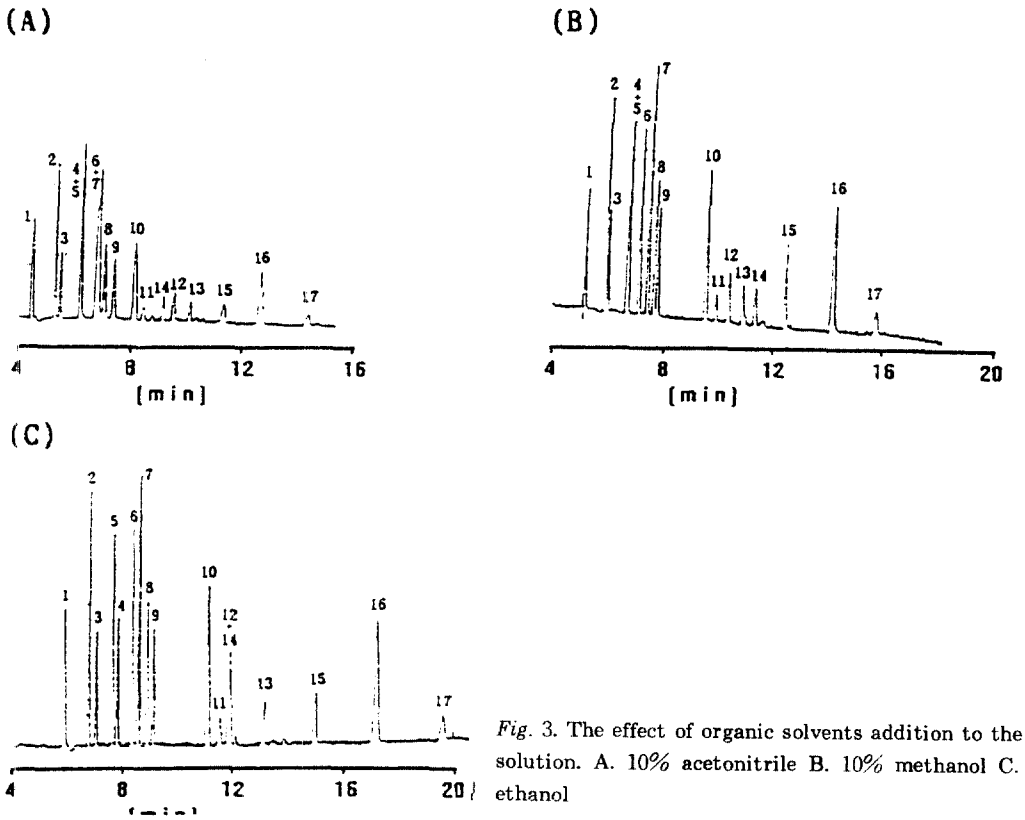

Fig. 3. The effect of organic solvents addition to the SDS solution. A. 10% acetonitrile B. 10% methanol C. 10% ethanol

buffer resolved all organic constituents in test mixture except for DNN and DEP. The organic solvents are expected to exhibit the same effect as urea, i.e., the additions of organic solvents to buffers change the electroosmotic velocity making longer analysis time and distribution ratio of analyte between aqueous layer and micelle.[18] However, unlike the urea, organic solvents had changed the order between the constituents. In fact each solvent, produces a different, eluting order for their constituents. Therefore, it may be possible to vary the type of organic solvent and use the change in elution time as a way to identify explosive constituents and we propose to achive desired separation of constituents in explosive mixtures.

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