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Capillary zone electrophoresis with indirect UV detection of organic anions using 2,6-naphthalenedicarboxylic acid

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Abstract

2,6-Naphthalenedicarboxylic acid (NDC) was characterized as a carrier electrolyte for a separation of organic anions by capillary zone electrophoresis (CZE) with indirect UV detection. Parameters which influence CZE separations such as concentration of NDC, electrolyte pH and various electroosmotic flow modifiers were investigated. The sensitivity obtained with the NDC electrolyte was five times higher than with phthalate, which is commonly used for the separation of organic anions. This method was applied to the determination of organic anions in the ambient air.

1. Introduction

The analysis of inorganic and organic anions using capillary zone electrophoresis (CZE) with indirect detection continues to be of interest [1–16]. Most published work is based on the indirect absorption detection, because UV–visible absorption detectors are still the most popular due to their versatility and simplicity, and because they are supplied with every commercial CE system [17]. This method of detection has long been used in single-column ion chromatography [18].

Many investigations have been undertaken to achieve high-sensitivity detection by the selection of appropriate electrolyte composition for indirect absorption CZE [1,3–6,9,15,16]. The optimization of CZE with indirect UV detection consists of selection of a carrier electrolyte with

large molar absorptivity and effective mobility similar to that of the sample ions [1]. The correlation between peak response and carrier electrolyte molar absorptivity in capillary electrophoresis has been documented by Jandik and Jones [4]. Choice of electrolyte components must be carefully considered, because mis-matched ionic mobilities of the carrier electrolytes and sample ions produce peak fronting or tailing [1,3]. Various chromophore-containing electrolytes based on chromate, pyromellitate, benzoate, phthalate and other aromatic carboxylic acid salts have been characterized for the analysis of inorganic and organic anions by CZE in many real samples [3–15].

The characterization of 2,6-naphthalenedicarboxylic acid (NDC) as a promising carrier ion for separation of less mobile anions by CZE with indirect UV detection is reported in this paper. NDC absorbs strongly in the UV region and is suitable as an electrolyte as well, because its

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electrophoretic mobility when fully ionized closely matches many organic anions.

Application of the NDC-based electrolyte is demonstrated on organic anion analysis in the ambient air.

2. Experimental

2.1. Instrumentation

Measurements were carried out on a P/ACE 2100 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA), equipped with a UV detector (filters for 200, 214, 254 and 280 nm), an autosampler and a temperature-controlled fluid-cooled capillary cartridge. An IBM computer (PS2/70) and Gold software v. 7.11 (Beckman Instruments) were used for instrument control and for data collection and processing. A 57.0 cm long (50.0 cm to the detector cell) \times 75 μ m I.D. fused-silica capillary (Beckman Instruments) was used. The temperature of a capillary was kept at $25 \pm 0.1^\circ\text{C}$. The applied voltage was 20 kV using the negative power supply and all injections were achieved by applying 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa) pressure for 10 s, unless otherwise noted.

Glass vials (5 ml) and 100- μ l polyolefin microvials were used for electrolytes and samples, respectively. All vials were rinsed with deionized water and dried prior to use.

A Beckman pH meter with a combination electrode was used to measure the pH of electrolytes.

Molar absorptivities of aqueous 2,6-naphthalenedicarboxylate (sodium form) and potassium hydrogenphthalate solutions were determined using a CARY I UV-Vis spectrophotometer (Varian Australia, Springvale, Australia) with 1-cm path quartz cells.

2.2. Reagents

All chemicals were obtained from either Aldrich (Milwaukee, WI, USA) or Fisher Scientific (Ottawa, Canada) at the highest purity available, and were used without additional purification.

All solutions, electrolytes and standards were prepared using deionized water (18 M Ω cm resistance) obtained by treating the tap water using reverse osmosis and ion exchange (Millipore, Model RO 20 and Model SuperQ).

2.3. Electrolytes and procedures

All NDC-based electrolytes were prepared each day from the stock solution which contained 20 mM NDC and 50 mM NaOH, and from 10 mM solution of tetradecyltrimethylammonium bromide (TTAB) as an electroosmotic flow (EOF) modifier. Other EOF modifiers such as tetrabutylammonium hydroxide (TBAOH), dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB) were also tested in this study. The pH was then adjusted with NaOH or sulphuric acid as required.

The phthalate electrolyte used in this study contained 5 mM potassium hydrogenphthalate, 2.0 mM boric acid and 0.5 mM TTAB.

In order to compare mobility of NDC with respect to phthalate, both carrier electrolyte anions were injected into a 5 mM borate buffer with 0.5 mM TTAB at pH 8, and migration time of NDC relative to phthalate was measured by direct UV detection at 254 nm.

All electrolyte solutions were filtered through a 0.22- μ m syringe PTFE membrane filter (Nalgene Brand Products, Rochester, NY, USA) and degassed by creating vacuum inside the syringe.

Separations were carried out using a method that consisted of a 1 min rinse of capillary with used electrolyte immediately prior to injection. At the beginning of each experimental day, the capillary was pretreated with 0.1 M NaOH for 10 min, then rinsed with deionized water (5 min) and with the used electrolyte (2 min).

2.4. Quantitation procedure

The mixed anion stock solution was diluted to produce working standard solutions at four different concentrations within the range 0.2–10 μ g/ml. Calibration graphs were plotted based on

the linear regression analysis of the corrected peak area.

Identification of individual ions in extracts of ambient air was based on the comparison of migration times of analytes with those of standard solutions. Also, all migration times were normalized to that of carbonate or bromide in order to obtain reproducible results. Bromide was added to the CE electrolyte as TTAB (EOF modifier) and acted as a negative reference peak. Some samples were spiked with standard solution. Ion chromatography with different selectivity than CE was also used for identification of some ions.

Detection limits were defined as three times the standard deviation of 18 replicate analyses of a standard with concentration equal to about ten times the estimated detection limit, the latter being the concentration giving a signal-to-noise ratio of 3.

2.5. Ambient air extracts

Ambient air constituents (particles, liquids and gases) were sampled by using the annular denuder/filter pack system [19,20].

The gaseous inorganic and organic acids, either collected during sampling or formed by oxidation on the carbonate coated denuders were extracted with 10 ml of deionized water and analyzed by ion chromatography [20].

IC-H Solid-phase extraction (SPE) disks (Alltech, Deerfield, IL, USA) were used to reduce of amount of carbonate presented in the extract for CZE analysis. Before applying sample, the disk was pre-conditioned by passing 10-ml of deionized water. Then the sample (approximately 3 ml) was passed through the disk at a flow-rate of less than 1 ml/min. The first 1 ml of the eluate was discarded and the remaining eluate was collected for analysis [21].

3. Results and discussion

The UV absorption spectrum of the NDC solution from 220 to 350 nm shows an absorption maximum at 235 nm and 284 nm with a large

molar absorptivity at 284 nm ($\epsilon = 11\,000\text{ l mol}^{-1}\text{ cm}^{-1}$). Because of the availability of a 280-nm filter in the used CE instrument, 280 nm was chosen as a representative detection wavelength throughout this work. NDC has a molar absorptivity higher by a factor of about 8 than phthalate (NDC: $\epsilon = 7667\text{ l mol}^{-1}\text{ cm}^{-1}$ and $10\,020\text{ l mol}^{-1}\text{ cm}^{-1}$ at 254 nm and 280 nm, respectively; phthalate: $\epsilon = 1350\text{ l mol}^{-1}\text{ cm}^{-1}$ and $600\text{ l mol}^{-1}\text{ cm}^{-1}$ at 254 nm and 280 nm, respectively). Lower detection limits can be therefore achieved. The strong UV absorbance of NDC at 280 nm allows also to detect anions with absorption in the wavelength range up to 250 nm with reasonable sensitivity by the indirect detection (i.e. phthalate and benzoate). As expected, the mobility of NDC is slightly lower than the mobility of phthalate. The relative migration time of NDC with respect to phthalate obtained at pH 8 and with direct detection at 254 nm was 1.07 and indicates its suitability as an electrolyte for separation of organic anions. NDC has also little adsorptive interaction with the column walls, as indicated by its very sharp elution peak in the direct mode. Hence, this compound is suitable for indirect detection.

3.1. Optimization of separation

The effects of various parameters such as concentration of NDC, electrolyte pH and various EOF modifiers were investigated to determine the optimum conditions of CZE separation. A standard solution of anions of interest at concentration $4\text{ }\mu\text{g/ml}$ was used in this study.

The optimization of the carrier ion concentration and ionic strength of electrolyte is very crucial in CZE with indirect UV detection [5,9]. Low concentration of carrier ion results in a better signal-to-noise ratio [18], but the overloading of system occurs due to the decrease of sample ion to carrier ion concentration ratio. The linear dynamic range decreases also. As the electrolyte concentration increases and consequently electrolyte conductivity rises, baseline noise due to additional Joule heating increases as well. This effect is much more significant with the indirect UV-detection electrolyte system than

with high UV-transparent electrolytes [22]. In this study, the increase of electrolyte concentration (NDC and sodium hydroxide) caused a rise of the current from $5.9 \mu\text{A}$ at 1 mM NDC (2.5 mM NaOH) to $36.2 \mu\text{A}$ at 8 mM NDC (20 mM NaOH) at 20 kV voltage. The optimum NDC concentration was found to be 2 mM which is the best compromise between peak separation, sensitivity and acceptable baseline noise level.

To investigate the influence of pH on the separation, measurements were made for the tested anions in 2 mM NDC-based electrolyte. The pH was varied in the range 6.1 to 11. A precipitate was formed in electrolytes below pH 6. There is no significant pH dependence for most of the tested anions. A decrease in migration time is observed for hydrogencarbonate (Fig. 1). The reason for this behaviour is the full deprotonation at pH 11. The increase of charge of one unit leads to a higher electrophoretic mobility, and the migration time decreases. The carbonate often present in the natural samples may complicate the separation of methanesulphonate or other anions less mobile than carbonate (Fig. 2A). Poor resolution of methanesulphonate and hydrogencarbonate ions at pH 8 can be improved by using the electrolyte at pH 11 (Fig. 2B).

Various EOF modifiers such as TBAOH, DTAB, TTAB [23] and CTAB [24] were tested. These compounds are electrostatically attracted to the silanol groups on the inner wall of the capillary, resulting in shielding the negative charge of the silica and thus directly influences the direction and magnitude of EOF [23]. The more hydrophobic was the EOF modifier, the shorter migration times were obtained due to more effective change of the silica–water interface structure resulting in a better suppression or reversal of EOF. No significant difference in the analysis speed with the long alkyl group modifiers such as TTAB and CTAB was observed. In this study TTAB was selected as the EOF modifier to obtain separation of anions within 5 min without diminishing the resolution between the peaks. A plateau of TTAB amount adsorbed on the silica surface is reached at 0.5 mM concentration, which results in negligible change in the magnitude of the reversed EOF.

On the basis of the results reported above, the electrolyte containing the 2 mM NDC, 5 mM NaOH and 0.5 mM TTAB at pH 8 or 11 was used for separation of organic anions. Fig. 3 shows a separation of selected mono- and dicarboxylic acid anions at pH 11. The order of separation is the expected one, because migra-

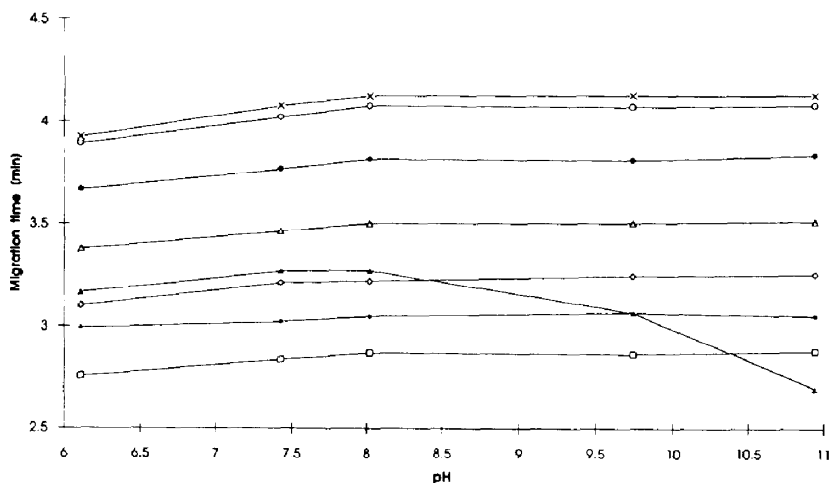


Fig. 1. Effect of the pH of the NDC electrolyte on the migration time of a $4 \mu\text{g/ml}$ anion standard mixture. \square = Formate; \blacklozenge = phthalate; \diamond = methanesulphonate; \blacktriangle = hydrogencarbonate; \triangle = acetate; \bullet = propionate; \circ = butyrate; \times = benzoate. Electrolyte: 2 mM NDC, 5 mM NaOH, 0.5 mM TTAB. See Experimental section for more details.

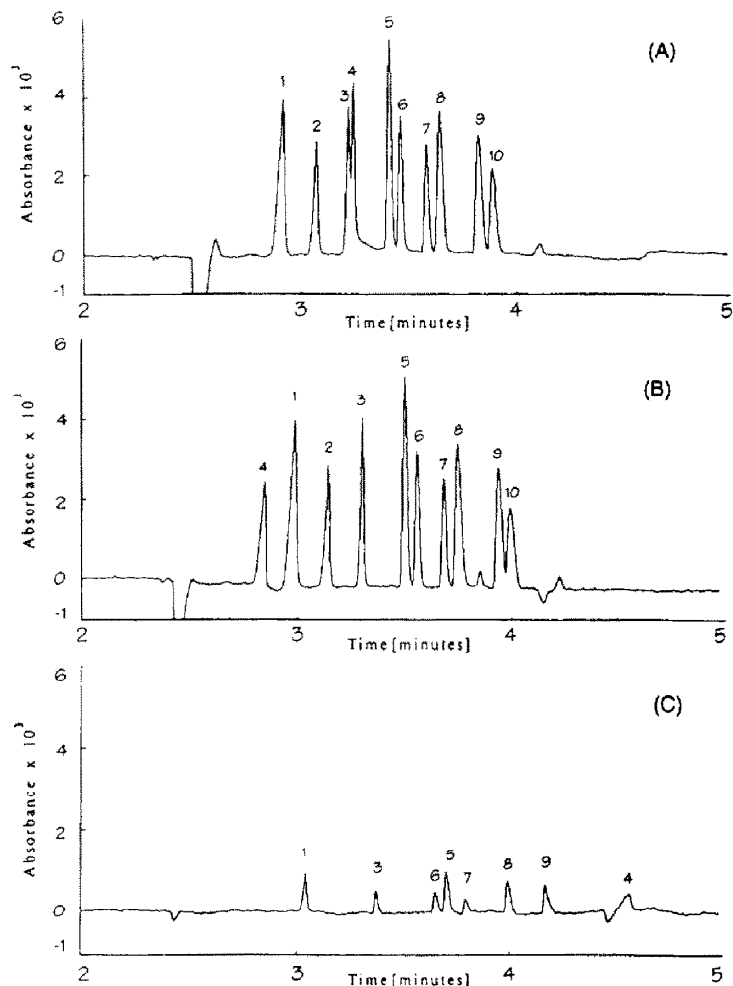


Fig. 2. Electropherograms of a 4 $\mu\text{g/ml}$ anion standard mixture obtained with the NDC electrolyte at pH 8 (A) and pH 11 (B), and with the phthalate electrolyte at pH 5.9 (C). Peaks: 1 = formate; 2 = phthalate; 3 = methanesulphonate; 4 = hydrogencarbonate or carbonate; 5 = acetate; 6 = chloroacetate; 7 = dichloroacetate; 8 = propionate; 9 = butyrate; 10 = benzoate. See Experimental section for more details.

tion time inversely relates to the equivalent conductance [5]. Another example of the capability of the NDC-based electrolyte as a powerful electrolyte is the separation of linear alkylsulphonates (Fig. 4).

3.2. Analytical performance

The precision of migration time and sample size are very important to obtain precise results in a quantitative analysis using CZE. With the electrolyte and instrument conditions estab-

lished, 18 injections of anion standard mixture at a concentration of 4 $\mu\text{g/ml}$ were made. The relative standard deviations (R.S.D.s) of migration times for the tested anions, shown in Table 1, were less than 0.5%, while the R.S.D.s of peak areas were less than 5%.

Quantitative determination using a pressure injection can be carried out on the basis of a linear relationship between peak area (or corrected peak area) and sample concentration over two orders of magnitude. Calibration graphs constructed for a mixed standard showed the

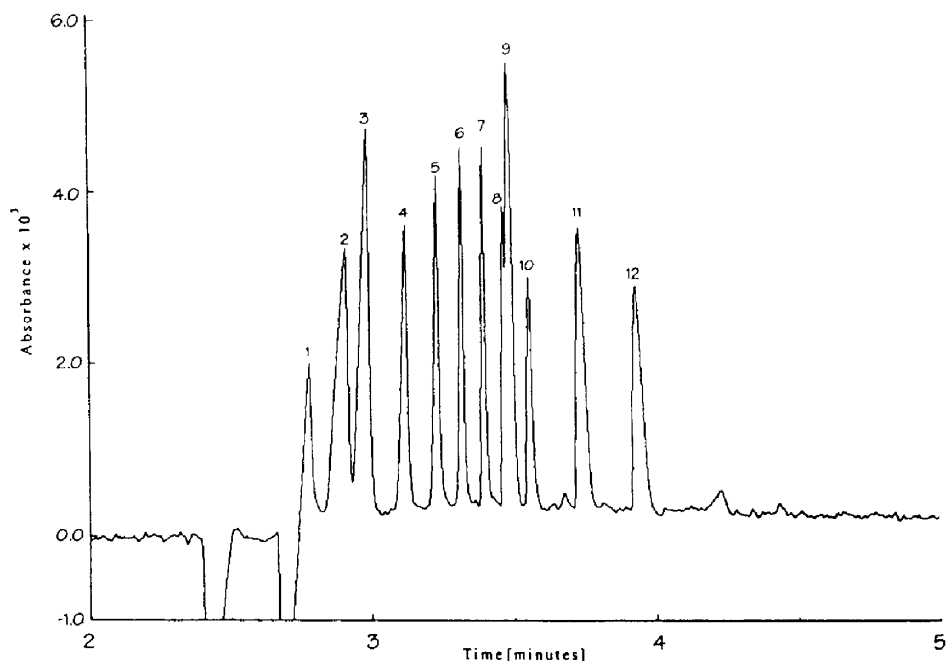


Fig. 3. Electropherogram of a 4 $\mu\text{g/ml}$ mono- and dicarboxylic anion standard mixture obtained with the NDC electrolyte at pH 11. Peaks: 1 = carbonate; 2 = fumarate; 3 = formate; 4 = glutarate; 5 = adipate; 6 = pimelate; 7 = suberate; 8 = azelate; 9 = acetate; 10 = sebacate; 11 = propionate; 12 = butyrate. See Experimental section for more details.

linear range extended from the detection limit to at least 20 $\mu\text{g/ml}$ with correlation coefficients ranging from 0.9989 to 0.9999 for all tested anions (Table 1). It should be mentioned that,

although the quantitation is possible at high sample concentration, the peak becomes broad and the resolving power is diminished. The NDC concentration should be increased when the

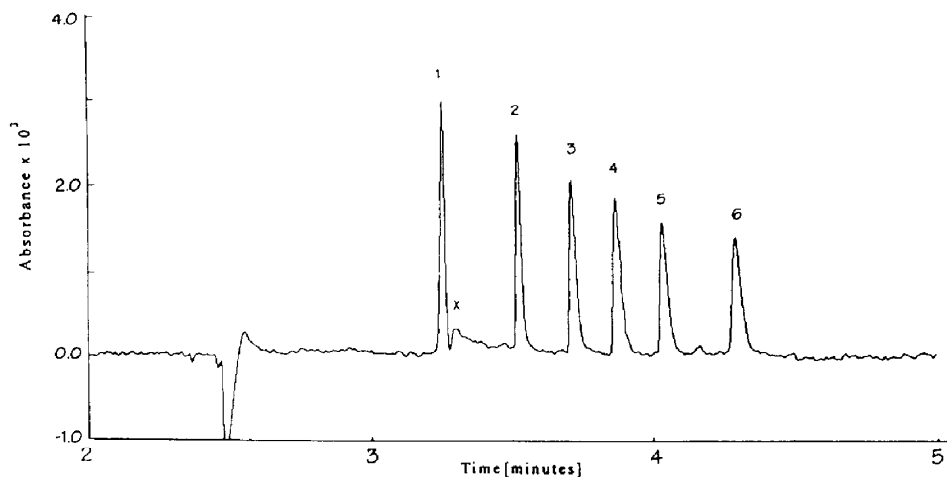


Fig. 4. Electropherogram of a 4 $\mu\text{g/ml}$ alkylsulphonate standard mixture obtained with the NDC electrolyte at pH 8. Peaks: 1 = methanesulphonate; 2 = ethanesulphonate; 3 = propanesulphonate; 4 = butanesulphonate; 5 = pentanesulphonate; 6 = hexanesulphonate; x = hydrogencarbonatc. See Experimental section for more details.

Table 1
Selected parameters of CZE using NDC

	R.S.D. ^a (%)		Correlation coefficient	Sensitivity ratio (NDC:KHP) ^b	Detection limit (ng/ml)
	Migration time	Area			
Formate	0.32	4.43	0.9999	5.40	133
Phthalate	0.30	4.52	0.9998	–	168
Methanesulphonate	0.31	4.20	0.9990	5.60	143
Acetate	0.38	3.42	0.9998	4.15	102
Propionate	0.45	3.39	0.9999	4.73	102
Butyrate	0.48	3.94	0.9995	3.93	118
Benzoate	0.49	4.00	0.9989	–	159

^a Relative standard deviation of 18 replicates of anion standard mixture at concentration 4 $\mu\text{g/ml}$.

^b The ratio of sensitivities (slopes of calibration curves) obtained with the NDC- and the phthalate-based electrolytes (KHP = potassium hydrogenphthalate).

analyte concentration is high or the injection time should be decreased.

The sensitivity defined as the peak area (or corrected peak area) per unit concentration can be easily compared for each anion. The sensitivity obtained with the NDC electrolyte is about five times higher than with the phthalate electrolyte (Table 1 and Fig. 2).

The detection limits of analytes (for 10-s pressure injection) using the described method are presented in Table 1. The results indicate that the proposed method gave the same or better detection limits than ion-exclusion chromatography commonly used for the separation and the determination of carboxylic acids [25].

3.3. Application

The proposed method was demonstrated on the determination of organic anions in aqueous extracts of atmospheric aerosol collected on thin PTFE filters [15] and in the extracts of ambient air collected by using of carbonate/glycerol-coated denuders. The determination of organic acids, in addition to the other inorganic acid species more commonly measured, is of great importance for a better understanding of the nature of acidity of ambient air.

Fig. 5A shows a typical electropherogram of

an ambient air extract obtained with the NDC-based electrolyte at pH 11. However, the presence of an excessive level of carbonate (from carbonate/glycerol-coated denuders) overlaps the following peaks and causes the peak broadening. Dilution of sample or removal of the carbonate before the analysis was necessary. Fig. 5B and C represent the separation of the same extract with the NDC-based electrolytes at pH 8 and 11, after passing it through a IC-H SPE disk to reduce the amount of carbonate. Recoveries of a group of carboxylic acids (formic, acetic, propionic, butyric and benzoic acids) in the presence of 0.1% sodium carbonate after using the IC-H SPE disks were found to be greater than 97%, except for benzoic acid, where recovery was greater than 92%. This "clean up" of carbonate allows determination of formate and other anions. There are some unidentified peaks with lower mobility than propionate present when the NDC electrolyte at pH 11 was used (Fig. 5A and C). This indicates that the denuder extract contains analytes which dissociate at pH higher than 8. The identification of these peaks will be the subject of future research.

4. Conclusions

This study has shown that CZE with indirect

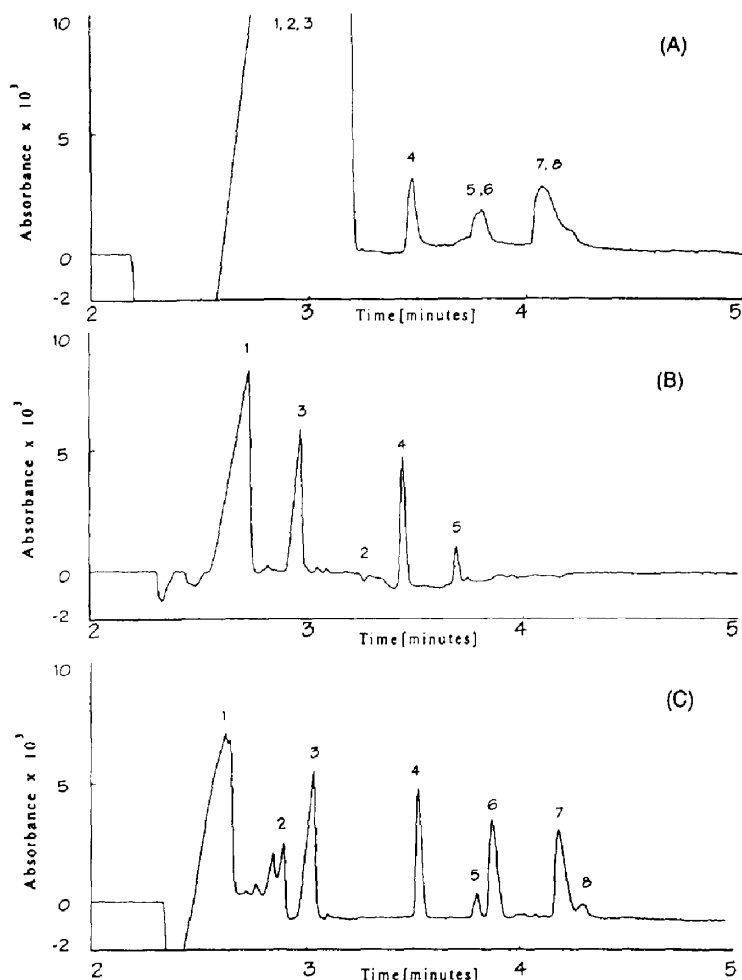


Fig. 5. Typical electropherograms of an ambient air extract collected by a carbonate/glycerol-coated annular denuder. (A) Untreated extract sample obtained with the NDC electrolyte at pH 11; (B, C) the same extract sample after treatment with IC-H SPE disk and obtained with the NDC electrolyte at pH 8 and 11, respectively. Peaks: 1 = unresolved inorganic anions; 2 = carbonate or hydrogencarbonate; 3 = formate; 4 = acetate; 5 = propionate; 6–8 = unidentified. See Experimental section for more details.

UV detection, employing NDC as the carrier electrolyte, is an effective method for the separation of organic anions.

The use of NDC as the carrier electrolyte has some advantages over other carriers such as phthalate: (i) UV absorption is at a longer wavelength (near 300 nm) and is very strong resulting in five times higher sensitivity; (ii) it allows for the detection of anions with absorption up to 250 nm by indirect detection; (iii) it

minimizes quantitation errors by reducing the interference from UV-absorbing substances, which might be present in real samples.

References

- [1] F. Foret, S. Fanali, L. Ossicini and P.J. Boček, *J. Chromatogr.*, 470 (1989) 299–308.
- [2] W.R. Jones and P. Jandik, *Am. Lab.*, 22, No. 9 (1990) 51–58.

- [3] J. Romano, P. Jandik, W.R. Jones and P. Jackson, *J. Chromatogr.*, 546 (1991) 411–421.
- [4] P. Jandik and W.R. Jones, *J. Chromatogr.*, 546 (1991) 431–443.
- [5] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445–458.
- [6] B.F. Kenney, *J. Chromatogr.*, 546 (1991) 423–430.
- [7] A. Nardi, M. Cristalli, C. Desiderio, L. Ossicini, S.K. Shukla and S. Fanali, *J. Microcol. Sep.*, 4 (1992) 9–11.
- [8] W. Jones, *J. Chromatogr.*, 640 (1993) 387–395.
- [9] M.P. Harold, M. Jo Wojtusik, J. Riviello and P. Henson, *J. Chromatogr.*, 640 (1993) 463–471.
- [10] P. Jandik and G. Bonn, *Capillary Electrophoresis of Small Molecules and Ions*, VCH, New York, 1993.
- [11] K.A. Hargadon and B.R. McCord, *J. Chromatogr.*, 602 (1992) 241–247.
- [12] D.L. Kelly and R.J. Nelson, *J. Liq. Chromatogr.*, 16 (1993) 2103–2112.
- [13] J.P. Romano and J. Krol, *J. Chromatogr.*, 640 (1993) 403–412.
- [14] J.B. Nair and C.G. Izzo, *J. Chromatogr.*, 640 (1993) 445–461.
- [15] E. Dabek-Zlotorzynska and J.F. Dlouhy, *J. Chromatogr. A*, 671 (1994) 389–395.
- [16] S.M. Cousins, P.R. Haddad and W. Buchberger, *J. Chromatogr. A*, 671 (1994) 397–402.
- [17] A.G. Ewing, R.A. Wallingford and T.M. Olefirowicz, *Anal. Chem.*, 61 (1989) 292A–303A.
- [18] H. Small and T.E. Miller, Jr., *Anal. Chem.*, 54 (1982) 462–469.
- [19] P. Koutrakis, J.M. Wolfson, J.L. Slatcr, M. Brauer, J.D. Spengler, R.K. Stevens and C.L. Stone, *Environ. Sci. Technol.*, 22 (1988) 1463–1468.
- [20] J.R. Brook, *Canadian Acid Aerosol Measurement Program, 1992–1993*, Atmospheric Environment Service, Toronto, 1993.
- [21] R. Saari-Nordhaus, L.M. Nair and J.M. Anderson, Jr., *J. Chromatogr. A*, 671 (1994) 159–163.
- [22] W. Beck and H. Engelhardt, *Fresenius' J. Anal. Chem.*, 346 (1993) 618–621.
- [23] X. Huang, J.A. Luckey, M.J. Gordon and R.Z. Zare, *Anal. Chem.*, 61 (1989) 766–770.
- [24] K. Altria and C. Simpson, *Anal. Proc.*, 23 (1986) 453–454.
- [25] V. Cheam, *Analyst*, 117 (1992) 1137–1144.