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Separation of carbonyl 2,4-dinitrophenylhydrazones by capillary electrochromatography with diode array detection

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Abstract

The applicability of capillary electrochromatography (CEC) with photodiode array detection for the analysis of carbonyl hydrazones is presented. The CEC separation of thirteen hydrazones was optimized by a systematic variation of conditions using a commercially available CE system and a 3-μm porous C_{18}-bonded silica capillary column. The separation profile obtained under optimal isocratic conditions (60% actonitrile–4% tetrahydrofuran–5 m*M* Tris, pH 8) is similar to those reported for gradient HPLC, with significant improvements in efficiency (to 150 000–250 000 theoretical plates/m) and analysis time (by a factor of 4). The retention time reproducibility is better than 0.2% (RSD) from run to run and 1% from day to day. The limits of detection for individual carbonyl hydrazones range between 0.1 and 0.5 μg/ml. Applications to ambient air and automobile exhaust are shown.

Keywords: Electrochromatography; Air analysis; Environmental analysis; Carbonyl compounds; Dinitrophenylhydrazones; Hydrazones

1. Introduction

Carbonyl compounds have received much attention in air pollution because of their toxicity and potential reaction with other pollutants to form carcinogens [1]. These pollutants are emitted by natural and industrial processes, motor vehicles and several other combustion sources. In the troposphere, these species are formed as intermediates in the photochemical degradation of hydrocarbons. The essential contribution of carbonyl compounds to the formation of radicals and ozone and their influence on the formation of photosmog is well known [2].

Because of the environmental importance of these compounds, selective and sensitive methods for the determination of volatile carbonyls in ambient air are needed. During the past two decades, different spectroscopic and chromatographic methods have been developed for measuring formaldehyde and other volatile aldehydes and ketones [3–9]. The most extensively used procedure is based on trapping of volatile carbonyls on solid adsorbents coated with 2,4-dinitrophenylhydrazine (DNPH) followed by analysis of the resulting hydrazones by high-performance liquid chromatography (HPLC) and UV absorbance detection [5–8]. The DNPH method is now widely applied in air monitoring programs, as recommended by the US Environmental Protection Agency (EPA) [10,11]. However, problems have recently become apparent with the DNPH method

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due to reaction of the reagent with ozone [12] and nitrogen dioxide [13] in air samples. In the case of ozone, several products are formed but they have not yet been identified. Nitrogen dioxide yields one product with DNPH, which has been identified as 2,4-dinitrophenyl azide (DNPA). These reaction products of both ozone and nitrogen dioxide may coelute with the formaldehyde hydrazone. In addition, HPLC consumes a lot of organic solvents and consequently creates another waste problem. For these reasons, interest continues in the development of new methods giving faster and more efficient separation with better resolution.

Recently, capillary electrochromatography (CEC) has been actively investigated [14–16]. It can be considered as a hybrid technique of micro-packed high-performance liquid chromatography (μ-HPLC) and capillary electrophoresis (CE), which couples the separating power of HPLC and the high efficiencies of CE. It offers all the advantages of a miniaturized separation technique, including low solvent consumption, small sample volume and low operational cost. It also offers the potential for retention mechanism and selectivity normally afforded by HPLC, but with an electrically driven flow which reduces the band broadening associated with pressure-driven parabolic flow profiles. In addition, electroosmosis provides other merits: no pressure drop is generated, and hence much smaller particles or longer separation columns than those in μ-HPLC can be used. The initial recognition of these advantages should be credited to Pretorius et al. [17], who reported that a significantly reduced plate height was achieved. The CEC technique was further pioneered by Jorgenson and Lukacs [18], and Knox and Grant [19,20]. The technological developments of CEC, including capillary column fabrication techniques, have been accelerating ever since [21]. Most research using CEC has been focused on pharmaceutical applications [14,22,23]. The potential of CEC in the food industry and biological analysis has also been demonstrated [24–26]. Attention has also been paid to evaluating the effectiveness of CEC for the analysis of environmental samples (polycyclic aromatic hydrocarbons, heterocyclics and explosives) [27–30]. A major goal of this work has been to explore the use of CEC technology for the separation of carbonyl-DNPH hydrazones. The CEC separation of thirteen to fifteen compounds with diode array detection (DAD) was developed by a systematic search for optimal conditions using a commercially available CE system and a micro-packed porous C_{18}-bonded silica capillary column. The applicability of this CEC method to the analysis of carbonyls in ambient air and automobile exhausts is fully demonstrated in the present report.

2. Experimental

2.1. Instrumentation

CEC studies were performed on a Beckman P/ACE 5510 CE system (Fullerton, CA, USA) equipped with a DAD system. The capillary columns packed with a 3-μm porous C_{18}-bondend silica particles were obtained from Unimicro (Pleasanton, CA, USA). The dimensions of these capillary columns were 27 cm (20 cm from the inlet frit to the detection window)×75 μm I.D. The applied voltage ranged between 10 and 25 kV, and the temperature was varied between 20 and 40°C. Unless stated otherwise, electrokinetic injections were performed at 10 kV for 1 or 10 s. Analyte peaks were detected at 360 nm while DAD scanning was from 200 to 400 nm.

Columns (either new or problematic with bubbles) were flushed with the mobile phase, using a manual syringe pump (Unimicro) to generate a moderate pressure of about 500 p.s.i. for at least 2 h prior to operation in the CEC system (1 p.s.i.=6894.76 Pa). At the beginning of each day's work, the capillary columns was equilibrated with fresh mobile phase by applying 4 kV for 15 min and then 15 kV for 10 min. Mobile phases were also changed electroosmotically, whenever needed. At the end of the day, both ends of the capillary were placed in water to avoid dissolution of its polyimide coating by acetonitrile in the mobile phase.

2.2. Chemicals

Individual DNPH-carbonyl hydrazones (100–1000 μg/ml) and a mixture of 13 carbonyls (30 μg/ml each) in acetonitrile were obtained from Supelco (Oakvile, Canada). Another mixture of 15 carbonyls

(15 μg/ml each) in acetonitrile was obtained from Radian (Austin, TX, USA). Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl, 50 m*M*, pH 8) and 2-morpholinoethanesulfonic acid (MES, 50 m*M*, pH 6) were purchased from Beckman (Mississauga, Canada). All other chemicals and solvents were purchased from Fisher (Ottawa, Canada) in the highest purity available, and they were used without further purification. The mobile phase was filtered through a 0.2-μm PTFE microdisc filter (Gelman Science, Montreal, Canada) and degassed for 30 min by ultrasonication before use. All standards before injections were dissolved in acetonitrile–water (1:1, v/v), unless otherwise stated. Ambient air and automobile exhaust samples collected on 2,4-DNPH coated cartridges were extracted with acetonitrile and diluted with water (1:1, v/v) prior to the CEC analysis.

3. Results and discussion

In CEC, separation is achieved by partitioning of the analytes between the mobile phase and the stationary phase. If the analytes are charged, the separation will also be governed by their differential electrophoretic mobilities in the mobile phase. Separation of neutral solutes such as the presents carbonyl hydrazones and other weakly basic and acidic compounds by CEC can be achieved with typical reversed-phase HPLC mobile and stationary phases. Therefore it should be straightforward, in principle, to transfer HPLC methods for such compounds directly to CEC and exploit the higher separation efficiency and shorter analysis times. However, since the surface properties of the stationary phase influence not only the selectivity but also the electroosmotic flow (EOF) in CEC, a systematic investigation of the effects of mobile phase and stationary phase variation on retention and selectivity is necessary for optimal performance [31].

Optimization of the separation conditions was achieved through testing the retention behavior of a mixture of thirteen 2,4-DNPH-carbonyl hydrazones (Table 1) on a commercially available micro-packed porous C_{18}-bonded silica column. The effect of various parameters such as mobile phase composition (percentage of organic solvent, pH, concentration of buffer solution), temperature, applied voltage and injection volume were optimized to achieve the best separation, the highest sensitivity and the shortest analysis time.

Table 1
Names and numbers of 13 carbonyl hydrazones for use in Figs. 1 and 2 only

Compound	No.	Compound	No.
Formaldehyde-DNPH	1	2-Butanone-DNPH	8
Acetaldehyde-DNPH	2	Butyraldehyde-DNPH	9
Acetone-DNPH	3	Benzaldehyde-2DNPH	10
Acrolein-DNPH	4	Valeraldehyde-DNPH	11
Propionaldehyde-DNPH	5	*p*-Tolualdehyde-DNPH	12
Crotonaldehyde-DNPH	6	Hexaldehyde-DNPH	13
Methacrolein-DNPH	7		

3.1. Effect of organic solvent content

In an initial analysis of carbonyl hydrazones by CEC, the effect of acetonitrile content (40–80%) on the separation selectivity was investigated while keeping the sodium tetraborate concentration of 1 m*M* and pH of 9 constant. As expected from changes in the partition coefficient, the retention times and retention factors (k') decreased for all analytes with an increase in content of acetonitrile in the mobile phase (data not shown). Under an applied voltage of 15 kV and a temperature of 20°C, the linear velocity of the EOF (measured by using acetone as the unretained marker) increased from about 0.8 to 1.1 mm/s with increasing acetonitrile concentration from 40 to 80%. This behavior agrees with most published reports [32]. Taking both resolution and speed for the majority of analytes into account, 60% acetonitrile was chosen for use in all subsequent experiments. As shown in Fig. 1a however, acrolein (3) coeluted with acetone (4), methacrolein (7) and 2-butanone (8) with butyraldehyde (9), and *p*-tolualdehyde (12) with hexaldehyde (13). Separation of analytes 3, 4 and 5 could be achieved when the mobile phase contained only 40% acetonitrile. Using such a low content of acetonitrile unfortunately resulted in both a large increase of retention times for all peaks and a decrease of detectability due to poor solubility of the hydrazones in the mobile phase. In addition, bubble formation became more problematic.

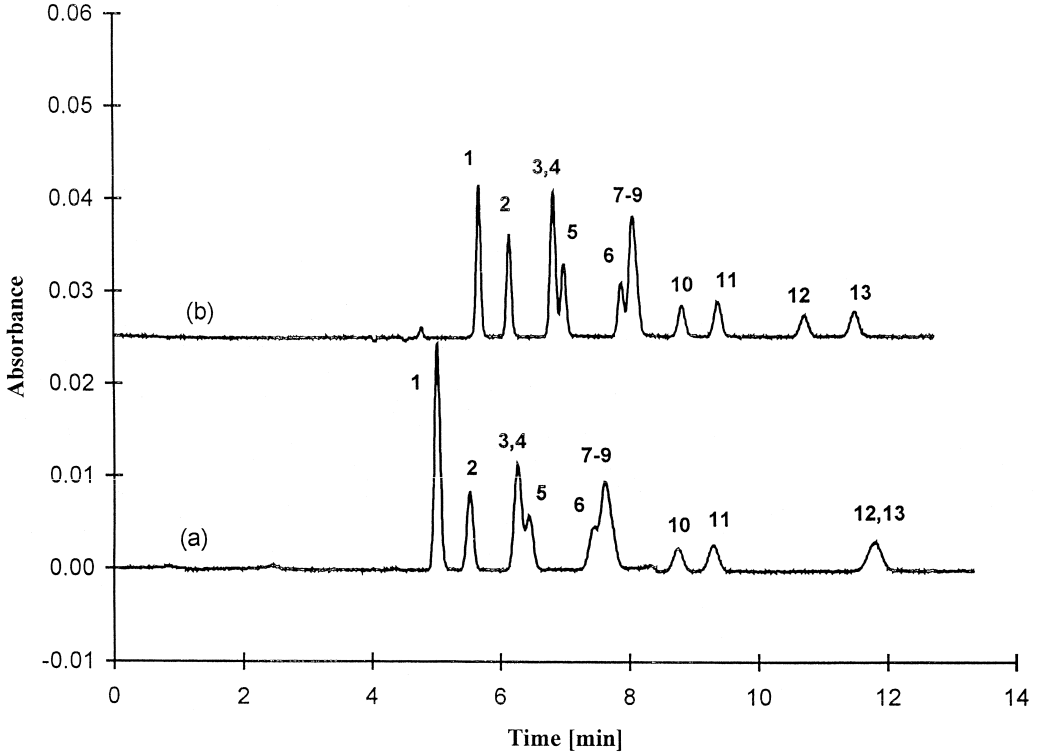

Fig. 1. CEC separation of carbonyl hydrazones using (a) 60% acetonitrile–1 m*M* borate (pH 9) and (b) 60% acetonitrile–4% THF–1 m*M* borate (pH 9) mobile phases. Conditions: column, 27 cm (20 cm effective length)×75 μm packed with 3-μm porous C_{18}-bonded silica particles; voltage 15 kV; detection: 360 nm (scan 200–400 nm); temperature 20°C, injection 10 kV for 1 s. The peaks in order of elution (15 μg/ml dissolved in 100% acetonitrile) are listed in Table 1.

Using a mobile phase consisting of 1 m*M* sodium tetraborate in 60% acetonitrile under 15 kV at 20°C, the theoretical plate number per meter varied between 55 000 and 90 000 for the carbonyl hydrazones. These plate numbers (N) were calculated according to the formula $N = 5.54\ (t_R/w_{1/2})^2$, where t_R is the retention time of the analyte, and $w_{1/2}$ is the width of the peak at the half-height, before normalized per meter of column length. Further improvement in the separation efficiency (plates number per meter up to 180 000) was achieved through the addition of 4% tetrahydrofuran (THF), as shown in Fig. 1b. The addition of THF allowed the more hydrophobic hydrazones to partition better between the stationary and mobile phases. Also, the resolution of the following peaks was improved: acrolein–acetone (3/4) and propionaldehyde (5); crotonaldehyde (6) and methacrolein–2-butanone–butyraldehyde (7–9); *p*-tolualdehyde (12) and hexaldehyde (13). This improvement was accompanied (but not caused) by a slight reduction in EOF velocity from 0.9 to 0.8 mm/s.

3.2. Effect of pH and buffer concentration

The effect of pH on the CEC separation of carbonyl hydrazones was investigated with various mobile phases that contained 60% acetonitrile–4% THF and 1 m*M* of different buffers to cover a pH range of 6–9. The buffers were MES for pH 6, Tris–HCl for pH 8 and sodium tetraborate for pH 9. The pH of each buffer was measured in the aqueous phase before mixing with the organic solvents. As in CE, increasing the pH of the mobile phase results in an increased EOF (0.4 mm/s at pH 6 and 0.7 mm/s at pH 8) due to dissociation of the surface silanol groups. A small increase in speed was indeed observed. However, this effect diminished above pH

8 resulting in a relatively constant EOF (0.8 mm/s at pH 9). Since the studied analytes are neutral, the pH of the mobile phase does not significantly affect their selectivity within the pH range of 6–9.

Due to frequent problems of bubble formation with mobile phases containing sodium tetraborate, a Tris–HCl buffer with lower conductivity was chosen in further experiments [33]. The increase of buffer concentration (1–5 m*M*) resulted in an improvement in the peak shape due to the stacking effect. This, however, came at the expense of a small increase in migration times of the hydrazones due to a decrease in the EOF caused by compression of double layer [34,35]. Since low buffer concentration can result in poor retention time reproducibility, caused by the low capacity of the buffer which in turn leads to ion

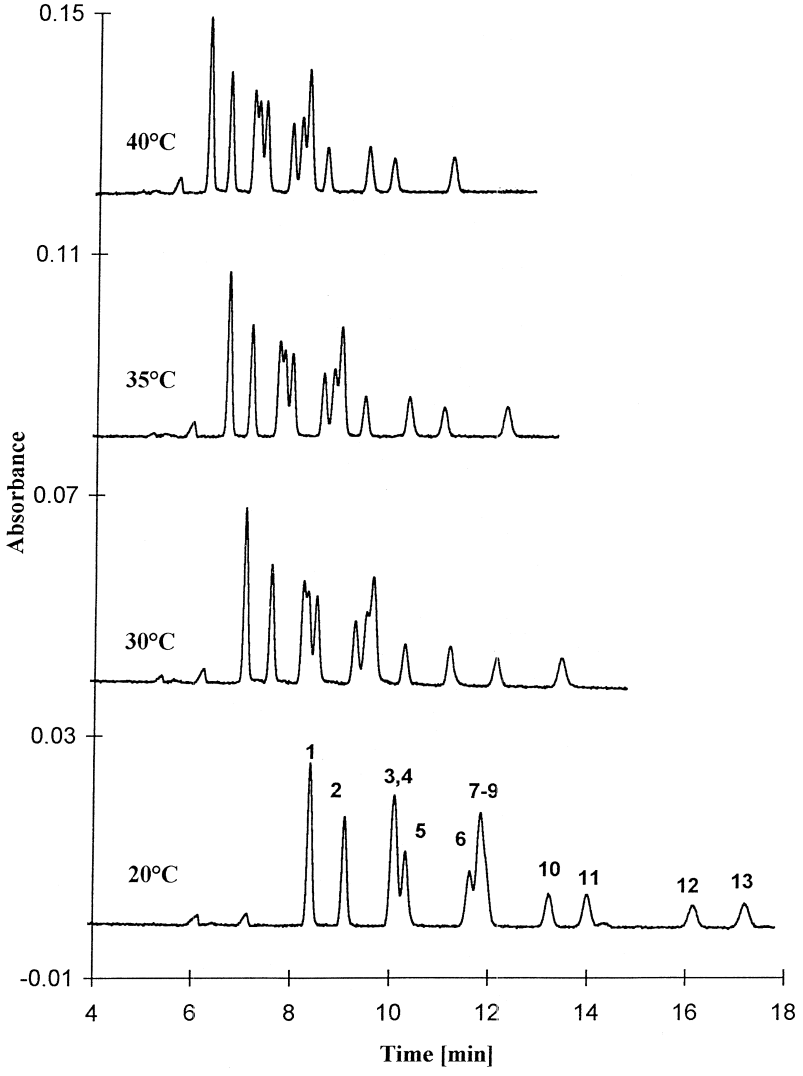

Fig. 2. Effect of temperature on the CEC separation of carbonyl hydrazones. Conditions: mobile phase, 60% acetonitrile–4% THF–5 m*M* Tris–HCl (pH 8); voltage, 15 kV; detection: 360 nm (scan 200–400 nm); injection 10 kV, 10 s. The peaks in order of elution dissolved in acetonitrile–water (1:1, v/v) are listed in Table 1.

depletion and pH gradients [33], a mobile phase containing 5 m*M* Tris was chosen for use in further experiments.

3.3. Effect of temperature and separation voltage

Another factor that had significant effects on the separation of carbonyl hydrazones was temperature. As can be seen in Fig. 2, both an improvement in the separation resolution and a reduction of retention times were obtained with higher temperatures. An increase in temperature from 20 to 40°C at 15 kV resulted in a 45% reduction in analysis time, while the EOF velocity increased only from about 0.50 to about 0.65 mm/s. An increase in temperature resulted in increased EOF due to lower electrolyte viscosity. The decrease migration times of hydrazones were both due to changes in the viscosity and an enhancement of mass transport at higher column temperatures which is similar to that observed in reversed-phase HPLC [36]. The selectivity of separation was slightly affected. A temperature of 35°C was chosen for subsequent experiments as it gives the best compromise between resolution and run time with an acceptable level of baseline noise.

The influence of the applied voltage on the current, the efficiency and the analysis time among the carbonyl hydrazones were next evaluated using a mobile phase of 5 m*M* Tris in 60% acetonitrile–4% THF at 35°C. As expected, a higher voltage reduced the analysis time by increasing the linear velocity from 0.4 mm/s at 10 kV up to 1.1 mm/s at 25 kV, whereas the current varied between 2.5 μA at 10 kV and 5.0 μA at 25 kV. Based on these results, the best separation efficiency with a short analysis time was obtained using 20 kV. The separation profile obtained

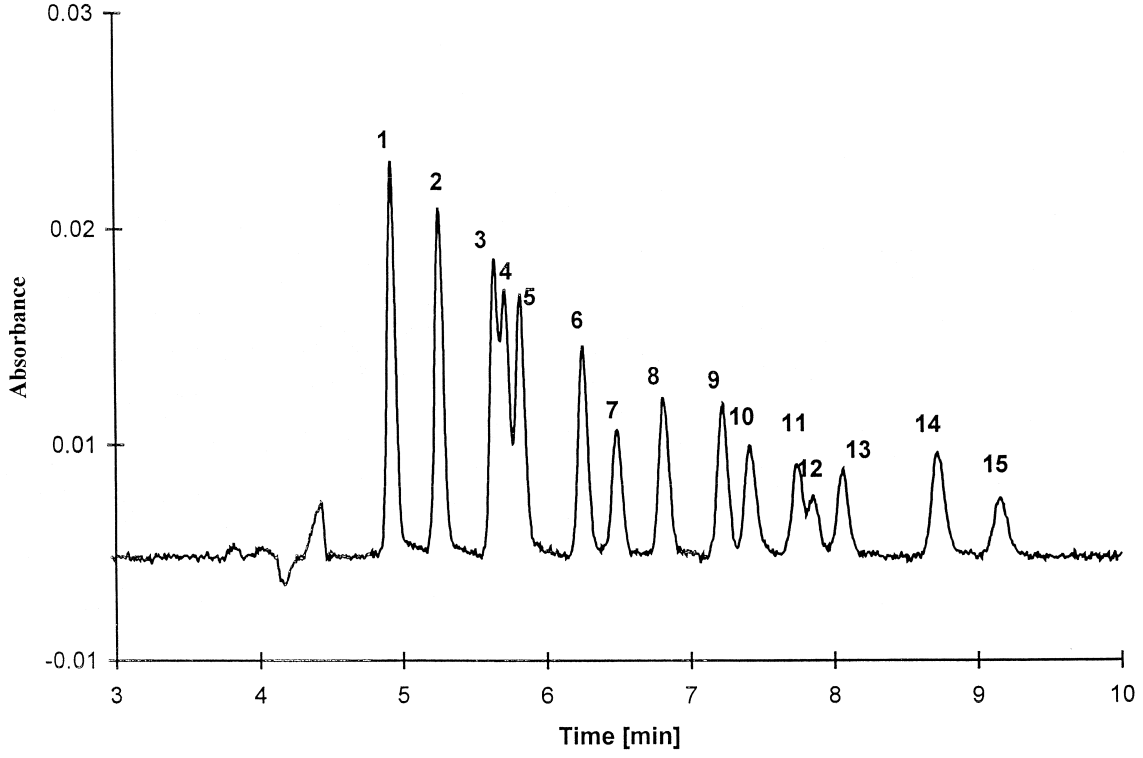

Fig. 3. CEC separation of 15 carbonyl hydrazones. Conditions: column, 27 cm (20 cm effective length)×75 μm packed with a 3-μm C_{18}-bonded porous silica particles; mobile phase, 60% acetonitrile–4% THF–5 m*M* Tris–HCl (pH 8); voltage, 20 kV; detection: 360 nm (scan 200–400 nm); temperature, 35°C, injection 10 kV, 10 s. The peaks correspond to: (1) formaldehyde, (2) acetaldehyde, (3) acetone, (4) acrolein, (5) propionaldehyde, (6) crotonaldehyde, (7) butyraldehyde, (8) benzaldehyde), (9) isovaleraldehyde, (10) valeraldehyde, (11) *o*-tolualdehyde, (12) *m*-tolualdehyde, (13) *p*-tolualdehyde, (14) hexaldehyde, (15) 2,5-dimethylbenzaldehyde.

under these optimal conditions is similar to those reported for HPLC, but with significant improvements in resolution, analysis time (by a factor of 4) and the theoretical plate number per meter (150 000–250 000) for the carbonyl hydrazones studied. Fig. 3 shows separation for a sample mixture of 15 hydrazones. With the exception of two pairs of hydrazones, acetone (3)–propionaldehyde (4) and *o*-tolualdehyde (11)–*m*-tolualdehyde (12), a baseline resolution is observed for all the hydrazones with an EOF velocity of 0.8 mm/s. Using a column longer than 20 cm could increase resolution of the studied hydrazones. The analysis is complete in 10 min under isocratic conditions, whereas a 35-min gradient HPLC elution would be required to obtain a similar separation pattern for the same mixture [11].

3.4. Analytical performance

Linearity of the developed CEC–DAD method was verified by analyzing carbonyl standards dissolved in acetonitrile–water (1:1, v/v), at six different concentrations in the range of 1–15 μg/ml. The plots were linear, with correlation coefficients ranging from 0.989 to 0.999. Using 10 kV injection for 10 s, the estimated detection limits (based on a signal-to-noise ratio equal to 3) for early eluting hydrazones (formaldehyde and acetaldehyde) were found to be 0.1 μg/ml and 0.5 μg/ml for the late eluting ones (benzaldehyde, hexaldehyde). Better detection limits can be obtained by increasing the injection time due to preconcentration of hydrazones at the beginning of the column. However, there was a corresponding decrease in separation efficiency with increasing of injection time because of a larger injected sample size. This effect was most pronounced for the totally unretained or weakly retained analytes (formaldehyde hydrazone), but it decreased with increasing analyte retention (or k'). As can be seen in Fig. 4, using a fixed voltage of 10 kV, for the

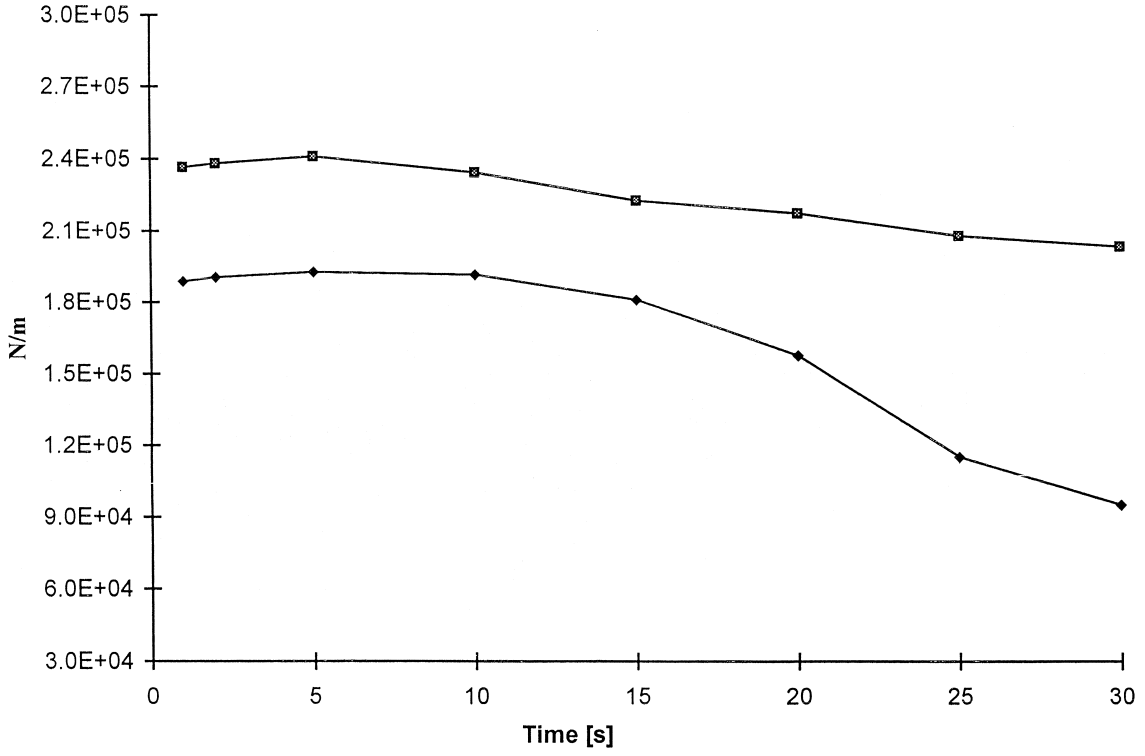

Fig. 4. Effect of injection time on the efficiency of formaldehyde (–♦–) and hexaldehyde (–□–) hydrazones are obtained from three replicate injections (RSD<5%). Conditions identical to Fig. 3.

Table 2
Retention time, area and efficiency reproducibilities[a]

Compound	t_R (min)	RSD (%)	Peak area	RSD (%)	N/m	RSD (%)
Run to run ($n=7$)						
Formaldehyde-DNPH (1)	4.72	0.08	81 265	3.34	189 900	3.94
Acetaldehyde-DNPH (2)	5.04	0.11	60 391	3.87	200 000	4.76
Propionaldehyde-DNPH (5)	5.61	0.17	49 788	4.14	214 500	3.54
Crotonaldehyde-DNPH (6)	6.03	0.09	37 666	3.88	228 900	3.79
Benzaldehyde-DNPH (10)	6.63	0.11	30 502	4.84	196 600	2.57
Hexaldehyde-DNPH (13)	8.65	0.14	25 362	4.48	200 200	2.72
Day to day ($n=7$)						
Formaldehyde-DNPH (1)	4.70	0.79	88 579	7.82	188 700	3.28
Acetaldehyde-DNPH (2)	5.02	0.90	64 426	7.66	195 200	3.58
Propionaldehyde-DNPH (5)	5.59	0.83	51 293	9.41	206 400	3.02
Crotonaldehyde-DNPH (6)	6.02	0.86	39 975	9.58	217 400	4.68
Benzaldehyde-DNPH (10)	6.61	1.04	32 654	9.15	210 200	7.93
Hexaldehyde-DNPH (13)	8.64	0.93	27 992	9.71	204 700	2.26

[a] Conditions identical to Fig. 3.

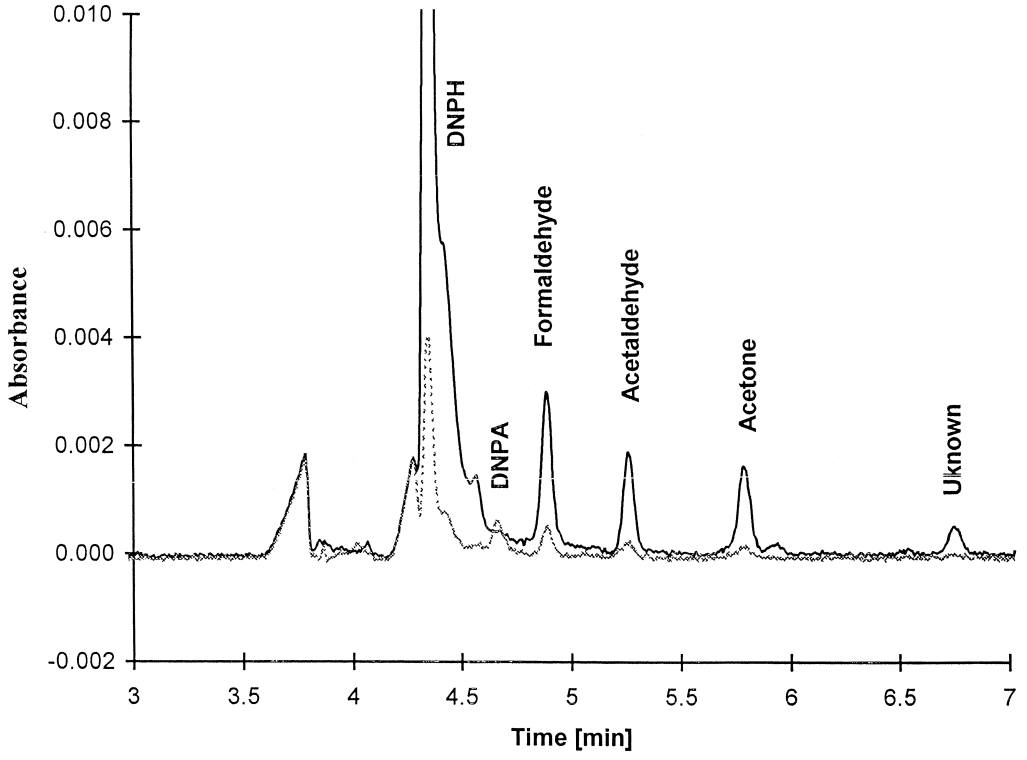

Fig. 5. CEC of an ambient air sample at detection wavelength $\lambda = 360$ nm (———) and 300 nm (- - -). Conditions identical to Fig. 3.

sample at concentration 5 μg/ml, the injection time can be as high as 20 s without a significant loss in column efficiency.

The short- and long-term reproducibility of the retention time, peak area and theoretical plates number per meter for some representative peaks are presented in Table 2. They are based on the data obtained from 25 runs over 7 days using a 3-μm porous C_{18}-bonded silica column and 60% acetonitrile–4% THF–5 m*M* Tris (pH 8) as the mobile phase. As expected, the relative standard deviations (RSDs) for most carbonyl hydrazones were generally low from run to run on the same day, when compared to typical day-to-day variations. Specifically, while the intra- and inter-day precisions of retention times are good, larger variation can be noticed in the peak area and theoretical plate number. The stability and reproducibility of EOF linear velocity were also assessed. Usually, the EOF velocity stabilized at about 0.84 mm/s (±0.01 mm/s for 20 kV) with intra- and inter-day reproducibilities of 0.2 and 0.6%, respectively.

3.5. Application to air and automobile exhaust samples

Example of the application of the developed CEC–DAD method to the analysis of carbonyls in 2,4-DNPH-cartridge sampled ambient air and automobile exhaust are shown in Figs. 5 and 6, respectively. The presence of certain carbonyl hydrazones in the extracts was confirmed by matching both their retention times and their UV spectra with those determined from pure standards. Most importantly, baseline separation of DNPA and the formaldehyde hydrazone (1) is observed, and they can be identified by dual-wavelength detection at $\lambda = 300$ nm and $\lambda = 360$ nm. Note that DNPA exhibits a stronger absorbance signal at 300 nm than at

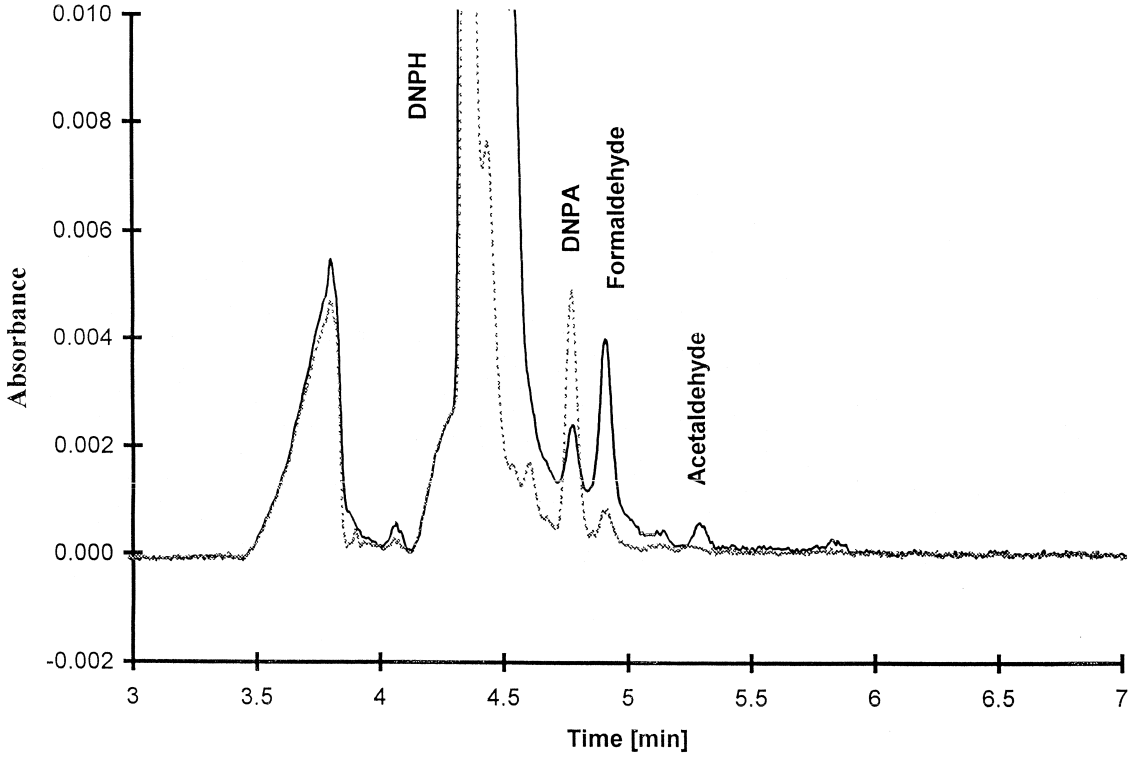

Fig. 6. CEC of an automobile exhaust sample at detection wavelength $\lambda = 360$ nm (———) and 300 nm (- - -). Conditions identical to Fig. 3, except for injection time of 20 s.

360 nm, while the converse is true for the formaldehyde hydrazone.

4. Conclusions

This work has fully demonstrated the development of a CEC–DAD method for the efficient analysis of carbonyl hydrazone mixtures. Efficiencies were much higher than those obtained in HPLC resulting in the short analysis time for a fairly complex mixture (<10 min). The preliminary applications of this method to ambient air and automobile exhaust samples show promise for future environmental studies. Research in our laboratories is now devoted to improving CEC separation by using smaller size packing materials and/or gradient elution. Gradient elution could increase peak capacity by providing better resolution of the earlier eluting hydrazones while still maintaining rapid elution of the later eluting compounds. Finally, cross-validation of the proposed method with an HPLC reference method will be undertaken.

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