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Determination of nitrophenols by capillary zone electrophoresis in a hydrodynamically closed separation compartment

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Abstract

The use of β-cyclodextrin (β-CD) and polyvinylpyrrolidone (PVP) as complexing agents to capillary zone electrophoretic (CZE) separations of a group of ten nitrophenols was investigated. The analytes were baseline resolved in one CZE run when PVP was present in the carrier electrolyte at a 2.5% (w/v) concentration. β-CD was less effective as it did not baseline resolve a complete group of the studied nitrophenols within an applicable concentration range (0–10 mmol/l). The separations were carried out in a 300-μm I.D. capillary tube made of fluorinated ethylene–propylene copolymer to enhance the load capacity of the separation system. Under these working conditions, a 100-nl sample injection volume resulted in 20–80 ppb concentration limits of detection for nitrophenols, using a photometric absorbance detector operating at a wavelength of 254 nm. The separation efficiencies for the analytes spanned from $1.6–2 \cdot 10^5$ theoretical plates per meter. Their migration times were reproducible not only in a within day but also in a day-to-day time frame (1% or less for a period of several weeks). This was achieved for a hydrodynamically closed separation compartment while minimizing fluctuations in the concentration of CO_2 in the carrier electrolyte. Reproducibility in the determination of nitrophenols was in the range of 1–5% for 1–6 ppm concentrations. Rain, drinking and process water samples served as matrices to assess the practical applicability of the elaborated CZE procedure. This procedure can be combined with isotachophoretic sample pretreatment to make its use possible in situations when nitrophenols are present in the samples at low ppb concentrations.

Keywords: Water analysis; Environmental analysis; Buffer composition; Hydrodynamically closed separation system; Phenols; Nitrophenols

1. Introduction

Nitrophenols currently need to be determined in various matrices linked with some industrial processes (production of dyes, pesticides and explosives) and, mainly, in matrices of environmental and ecotoxicological relevance [1–5]. At present, gas chromatography [1,2,6,7] and various alternatives of high-performance liquid chromatography [2,8–12] are preferred in the analysis of nitrophenols. Their ionogenic properties make capillary electrophoresis (CE) techniques analytical alternatives to these chromatography techniques. So far, however, only limited attention has been paid to this subject [13–19].

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Capillary isotachophoresis (ITP) was shown to be applicable to the separations of some nitrophenols [13,14]. For mononitrophenols, differences in their pK_a values were found to be suitable for separating them [13]. The same separation mechanism, using a low pH of the leading electrolyte, was effective for some dinitrophenols and picric acid [14]. Here, the analytes could be detected at ppt concentrations when performing the ITP separations in the spike mode with selective photometric detection at 405 nm. However, the close ionic mobilities and the very differing pK_a values of nitrophenols [20] make this approach less practical when the separation and/or determination of a larger number of nitrophenols is to be carried out.

Nitrophenols listed as priority pollutants by the US Environmental Protection Agency [4] were recently separated by capillary zone electrophoresis (CZE), together with other priority phenol pollutants [17]. The separation mechanism in this instance included differences in the ionic mobilities and pK_a values of phenols. However, it is apparent that this separation mechanism has only a limited use for nitrophenols [18,19]. Micellar separation systems offer another CE alternative in resolving priority phenol pollutants [16].

The aim of this work was to find separation conditions applicable to the CZE separation and quantitation of a group of ten nitrophenols of environmental relevance. Here, we investigated in detail the use of β-cyclodextrin (β-CD) and polyvinylpyrrolidone (PVP), as these agents had already been proved [18,19] to influence the effective mobilities of nitrophenols in a differentiating way. To enhance sample loadability onto the CZE column and, hence, reduce the concentration limits of detection (LODs) for the analytes, we carried out our experiments in a 300-μm I.D. capillary tube made of fluorinated ethylene–propylene copolymer (PEP). Key performance parameters characterizing the CZE separations under favourable resolution conditions were assessed. The elaborated CZE procedure was briefly tested in the analysis of practical samples (rain, drinking and process water) spiked with nitrophenols. The samples spiked at two concentration levels were analyzed either directly by CZE or in combination with ITP pretreatment.

2. Experimental

2.1. Instrumentation

CZE separations were carried out using a CS isotachophoretic analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) assembled in the single column mode. Its separation unit consisted of the following parts:

(i) A sample injection valve provided with a 100-nl internal sample loop (Villa-Labeco).

(ii) A 300-μm I.D. (650 μm O.D.) capillary tube made of fluorinated ethylene–propylene copolymer in which the CZE separations were performed. Its length from the injection valve to the detector was 300 mm, while the total length was 350 mm.

(iii) A counter-electrode compartment with a hydrodynamically closed connecting channel to the separation compartment (Villa-Labeco).

A Spectra 100 on-column photometric detector (Thermo Separation Products, San Jose, CA, USA) was used for detection at 254 nm. It was connected to a 486DX computer via a Unilab data acquisition unit (Fitek, Šal'a, Slovak Republic). ITP Win Software (version 2.15), obtained from KasComp (Bratislava, Slovak Republic), was used for data acquisition and processing.

The ITP equipment used for sample pretreatment prior to the final CZE analysis has been described elsewhere [21,22].

2.2. Chemicals and electrolyte solutions

The chemicals used for the preparation of the electrolyte solutions were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Merck (Darmstadt, Germany), Cyclolab (Budapest, Hungary), Fluka (Buchs, Switzerland) and Lachema (Brno, Czech Republic). Methylhydroxyethylcellulose 30 000 (m-HEC) was used as an additive to the leading and carrier electrolyte solutions. The 1% (w/v) aqueous stock solution of it was demineralized with the aid of Amberlite MB-1 mixed-bed ion exchanger (Serva). PVP was from Serva (PVP, K90). It was added to the carrier electrolyte solutions without further purification. Water from a Pro-PS water purification system

(Labconco, Kansas City, KS, USA) was used for the preparation of the solutions. The solutions were filtered through 0.8 μm syringe filters (Sigma) before use.

Absorption of CO_2 by the electrolyte solutions was minimized by keeping them in closed vessels in a desiccator over NaOH pellets. The electrolyte solutions in the electrode vessels of the apparatus were maintained in a closed environment using gas proof caps. The ambient pressure above the solutions in the electrode vessels was preserved through microcolumns packed with NaOH pellets, which were tightly connected to the vessels via Luer female connectors in the caps.

2.3. Samples

Stock aqueous solutions of the studied nitrophenols were prepared from chemicals obtained from the above suppliers. Their concentrations in the stock solutions were 100–200 ppm. The studied nitrophenols included: *o*-nitrophenol (o-NP), *p*-nitrophenol (p-NP), *m*-nitrophenol (m-NP), 2,4-dinitrophenol (2,4-DNP), 2,5-dinitrophenol (2,5-DNP), 2,6-dinitrophenol (2,6-DNP), 3,4-dinitrophenol (3,4-DNP), picric acid (2,4,6-TNP), *p*-nitro-*m*-cresol (p-N-m-C) and 4,6-dinitro-*o*-cresol (4,6-DN-*o*-C). Model samples were prepared by appropriately spiking aqueous solution of Na_2SO_4 (100 ppm) with nitrophenols from their stock solutions.

A rain water sample was obtained from the Slovak Hydrometeorological Institute (Bratislava, Slovak Republic) and a process water sample was kindly provided by Povodie Dunaja (Bratislava, Slovak Republic). Drinking (tap) water samples were collected in the laboratory in polyethylene sample containers.

3. Results and discussion

The pK_a values of the nitrophenols used in this study (see Section 2 for the list) ranged from 0.71 (picric acid) to 8.40 (*m*-nitrophenol) [20]. Therefore, CZE separations of them requires that the carrier electrolytes have high pH values. Under such conditions, the effective mobilities of nitrophenols are determined by the actual ionic mobilities [23,24]. These migration parameters of nitrophenols in aqueous solutions are close [20] and, consequently, resolution problems can be expected when their CZE separations are performed in this separation mode. This is illustrated by the electropherogram shown in Fig. 1.

Our previous work, aimed at finding suitable separation mechanisms for multicomponent mixtures of nitrophenols, revealed that β-CD and PVP, or their simultaneous use, in the carrier electrolyte offer promising alternatives in this respect [18,19]. Therefore, their use for the studied group of nitrophenols was investigated in detail in this work.

3.1. CZE separation of nitrophenols via host–guest complexation with β-CD

Assuming the formation of a 1:1 host–guest complex of nitrophenol (NP) with β-CD (C) at a high pH value (NP is present only in the anionic form), the following equilibrium is relevant:

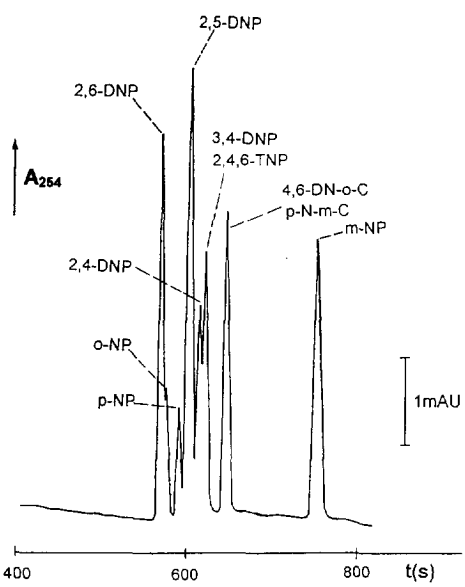

Fig. 1. CZE separation of nitrophenols according to ionic mobilities. The separation was carried out in electrolyte system No. 1 (Table 1) with a 100-μA driving current. The concentrations of the analytes in a model mixture were 5–10 ppm. For the abbreviations, see Section 2.

$$NP + C \rightleftarrows NPC \quad (1)$$

with the stability constant (K_{NPC})

$$K_{NPC} = \frac{[NPC]}{[NP][C]} \quad (2)$$

characterizing complex formation. [NPC], [NP] and [C] are symbols for the equilibrium concentrations of the species involved. From the point of view of the electrophoretic separations, this equilibrium process influences the effective mobility of nitrophenol ($\bar{m}_{NP}$) in a well known way [23,24] and, in our particular instance, we can write:

$$\bar{m}_{NP} = \frac{1}{1 + K_{NPC}c_C} \cdot m_{NP} + \frac{K_{NPC}c_C}{1 + K_{NPC}c_C} \cdot m_{NPC} \quad (3)$$

where c_C is total concentration of β-CD (it is assumed that [c]~c_C) and m_{NP} and m_{NPC} are actual ionic mobilities of the anionic form of nitrophenol and its complex, respectively. The effective mobility of the analyte and its migration time in CZE are interrelated [24] and, in our symbolics, we can write:

$$t_{NP} = \frac{L_d}{\bar{m}_{NP}E} \quad (4)$$

with t_{NP} being a symbol for the migration time of nitrophenol, L_d being the length of the capillary tube from the injection point to the detector and E being the electric field strength. Using Eq. (3), we can modify Eq. (4) into the form:

$$t_{NP} = \frac{L_d(1 + K_{NPC}c_C)}{(m_{NP} + K_{NPC}c_C m_{NPC})E} \quad (5)$$

which is convenient for interpreting the plots in Fig. 2.

From the dependence of the migration times of nitrophenols on the concentration of β-CD (Fig. 2), we can see that this complexing agent did not influence the migration times of 2,6-DNP and 2,4,6-TNP. Eqs. (4) and (5) enable us to conclude that the effective mobilities of these nitrophenols were determined by the actual mobilities of their anionic forms. Significant effects of β-CD on the effective mobilities of o-NP, 2,5-DNP, 4,6-DN-o-C and m-NP show that both free and complexed anionic forms contributed to the effective mobilities of this group of nitrophenols. For m-NP, in addition, a contribution of the uncharged acidic form ($pK_a = 8.40$) must also be taken into account. p-NP, p-N-m-C, 3,4-DNP and 2,4-DNP formed the strongest complexes with β-CD among the nitrophenols studied. For higher concentrations of β-CD, plots for these analytes were reaching plateau values, i.e., approaching conditions under which they exist mostly in complexed anionic forms (a contribution of the second term in the denominator of Eq. (5) to the migration time was the most significant).

From this relative classification of nitrophenols, we can see the significance of the position(s) of the nitro group(s). While nitro groups in the *p*-position favoured inclusions, two nitro groups in the *o*-posi-

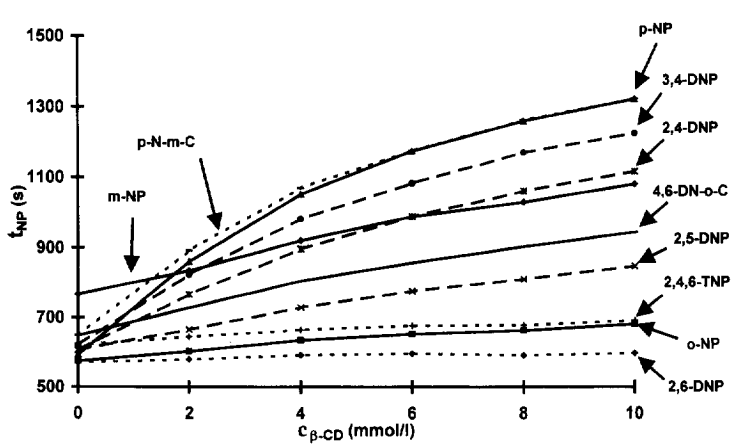

Fig. 2. Dependence of the migration times of nitrophenols on the concentration of β-CD in the carrier electrolyte. The measurements were carried out in electrolyte system No. 2 (Table 1) with a 100-μA driving current. For the abbreviations, see Section 2.

tion acted against this process (see the results for 2,6-DNP and 2,4,6-TNP). Other combinations of the positions of the substituents led to effects of intermediate significance and thus contributed to the apparent differentiating power of β-CD in terms of the CZE resolution. At some concentrations of β-CD in the range of 2–4 mmol/l, all of the nitrophenols studied could be resolved. However, at no concentration of β-CD could baseline resolution be achieved. Critical pairs in this respect are apparent from the plots in Fig. 2. At higher concentrations of β-CD in the carrier electrolyte, p-NP and p-N-m-C were not resolved at all. This resolution problem can be ascribed to very close actual ionic mobilities of their complexed anionic forms. the electropherogram in Fig. 3 shows that, under these conditions, the rest of the nitrophenols, with the exception of o-NP and 2,4,6-TNP, were baseline resolved.

3.2. CZE separations of nitrophenols in the presence of PVP in the carrier electrolyte

PVP is known to interact with a great variety of aromatic anions [25–29]. The interactions involved have been discussed extensively in the works of Molyneux and Vekavakayanondha [27,28]. For phenols, it is reasonable to assume that these include hydrogen bonding, dipole–dipole and induction forces and hydrophobic effects [27]. Formally, these interactions can be treated as a complex formation and, therefore, analogous relationships, as given for β-CD, are appropriate. For the dependence of the migration times of nitrophenols (t_{NP}) on the concentration of the polymer in the carrier electrolyte (c_P), we can assume the validity of the following relationship:

$$t_{NP} = \frac{L_d(1 + K_{NPP}c_P)}{m_{NP}E} \quad (6)$$

In this equation, K_{NPP} is a symbol for the stability (binding) constant of a given nitrophenol. The actual ionic mobilities of the nitrophenol–PVP complexes (m_{NPP}) were assumed to approach zero and in Eq. (6) (contrary to Eq. (5)), the second term in the denominator was neglected. This assumption corresponds with low values of the binding ratios of the phenol derivatives in the aqueous solutions of PVP [27]. The experimental plots shown in Fig. 4 generally conform with Eq. (6). These plots also show that PVP influenced the migration times (the effective mobilities) of nitrophenols in a differentiating way. A minimum retardation of the migration velocity due to the presence of PVP was observed for o-NP, m-NP and 2,5-DNP. Stronger effects of the polymer for p-NP, 2,4-DNP, 2,6-DNP and 2,4,6-TNP are apparent and the strongest interactions can be ascribed to 3,4-DNP, p-N-m-C and 4,6-DN-o-C.

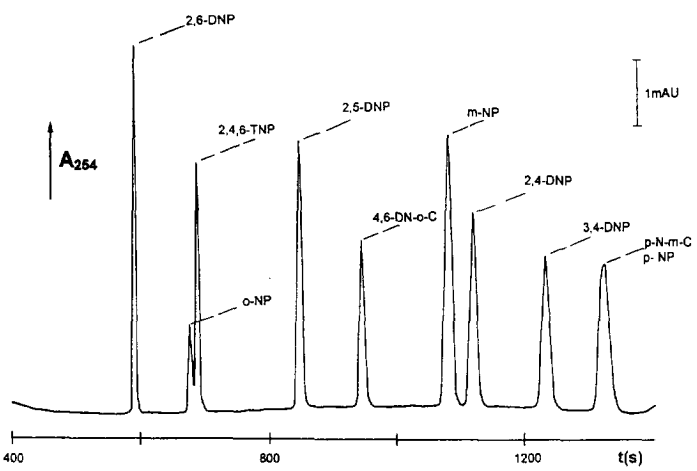

Fig. 3. CZE separation of nitrophenols based on host–guest complex equilibria with β-CD. The separation was carried out in electrolyte system No. 2 (Table 1) with a 10-mmol/l concentration of β-CD. The driving current was 100 μA. The same model mixture was used in the separation as was used in Fig. 1. For the abbreviations, see Section 2.

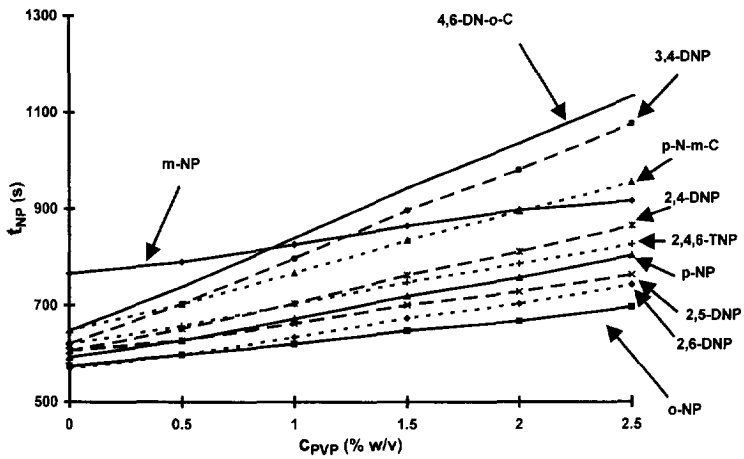

Fig. 4. Dependence of the migration times of nitrophenols on the concentration of PVP in the carrier electrolyte. The measurements were carried out in electrolyte system No. 3 (Table 1) with a 100-μA driving current. For the abbreviations, see Section 2.

From the plots shown in Fig. 4, it is clear that the presence of PVP in the carrier electrolyte improves the CZE resolution of the nitrophenols we are interested in. In this work, we favoured separation in an electrolyte system containing the polymer at a 2.5% (w/v) concentration. Under these conditions, the baseline resolution of nitrophenols was achieved, as illustrated by the electropherogram shown in Fig. 5.

A comparison of the data obtained for PVP and β-CD (see Figs. 2–5) shows that these complexing agents provide different separation selectivities for nitrophenols. This offers the possibility of optimizing their separation via the simultaneous use of both agents. Although this possibility had been shown already [18] in the CZE separation of nitrophenols, we did not study it in detail in this work.

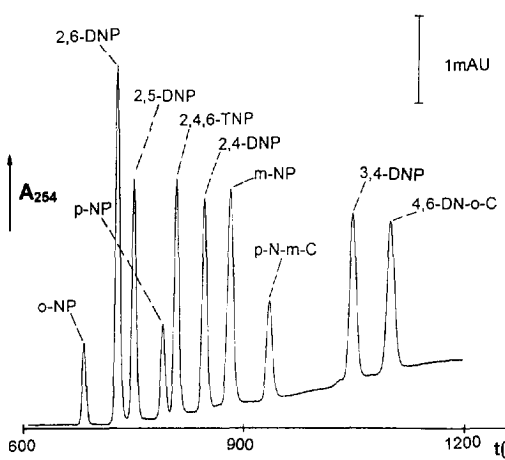

Fig. 5. CZE separation of nitrophenols based on their interactions with PVP. The separation was carried out in electrolyte system No. 3 (Table 1) with 2.5% (w/v) PVP. The driving current was 100 μA. The same model mixture was used in the separation as was used in Fig. 1. For the abbreviations, see Section 2.

3.3. Some separation performance parameters

Our experiments were carried out in a hydrodynamically closed separation compartment provided with a 300-μm I.D. capillary tube. The use of such a separation compartment requires that the electroosmotic flow is suppressed [23,24]. In our work, this was achieved by using a high molecular mass derivative of m-HEC in the carrier electrolyte (see Section 2). PVP may act in a similar way to m-HEC [23]. However, our experiments showed that by omitting m-HEC from the carrier electrolyte solution, a residual electroosmotic flow, manifested via reduced separation efficiencies of the analytes [24], occurred.

Using electrolyte system No. 3 (Table 1), which provided baseline resolution of nitrophenols, very reproducible migration times were typical not only in a within-day but also in a day-to-day time frame (Table 2). To achieve such day-to-day reproducibilities of the migration times of the analytes, careful preparation and storage of the carrier electrolyte

Table 1
Electrolyte systems

Parameter	CZE system no.		
	1	2	3
Carrier ion	Glycine	Glycine	Glycine
Concentration (mmol/l)	50	50	50
Counter-ion	BTP	BTP	BTP
Additive	m-HEC	m-HEC	m-HEC
Concentration (%, w/v)	0.2	0.2	0.2
Complexing additive	–	β-CD	PVP
Concentration	–	2–10 (mmol/l)	0.5–2.5 (%, w/v)
pH	9.1	9.1	9.1

	ITP system
Leading anion	Chloride
Concentration (mmol/l)	10
Counter-ion	BTP
Additive	m-HEC
Concentration (%, w/v)	0.2
pH	9.2
Terminating anion	Glycine
Concentration (mmol/l)	10
Counter-ion	BTP
pH	9.1

BTP = 1,3-bis[tris(hydroxymethyl)methylamino)propane; m-HEC = methylated hydroxyethylcellulose; β-CD = β-cyclodextrin; PVP = polyvinylpyrrolidone.

solution was essential (see Section 2). When appropriate precautions were not taken, fluctuations in the concentration of carbonate in the carrier electrolyte solution were unavoidable. This resulted in fluctuations in the migration times and in an uncontrollable decrease in the pH value via absorbed CO_2. The electropherograms shown in Fig. 6 illustrate these problems. Here, we can see that proportional shifts in the migration times due to increased conductivity of the carrier electrolyte were combined with selective

Table 2
Separation performance parameters for nitrophenols

Constituent	Reproducibility of migration time[a]		Separation efficiency[b]
	Within day ($n = 17$)	Day-to-day ($n = 59$)	
o-NP	0.31	0.98	196 000
m-NP	0.31	0.98	160 000
p-NP	0.32	1.00	204 000
2,4-DNP	0.32	0.98	184 000
2,5-DNP	0.29	0.99	190 000
2,6-DNP	0.36	1.00	203 000
3,4-DNP	0.33	0.95	203 000
2,4,6-TNP	0.28	0.98	203 000
p-N-m-C	0.31	0.95	174 000
4,6-DN-o-C	0.32	0.92	166 000

[a] Reproducibilities of the migration times are expressed as relative standard deviations.
[b] Separation efficiencies were calculated as the number of theoretical plates for a 1-m column.

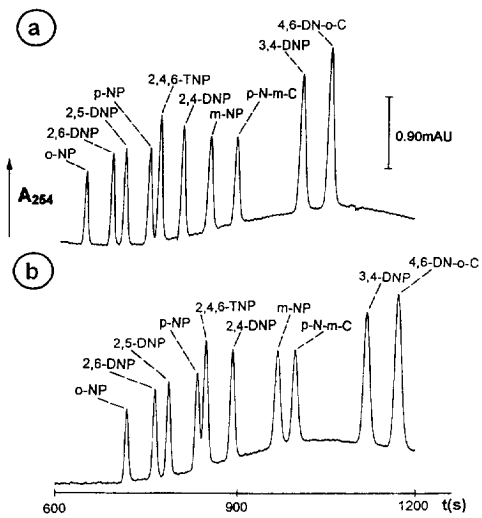

Fig. 6. Influence of CO_2 on the migration times of nitrophenols. The separations were carried out in electrolyte system No. 3 (Table 1) with 2.5% (w/v) PVP. The model mixture contained the analytes at 5–10 ppm concentrations. a = The separation compartment and the electrode vessels were filled with a freshly prepared solution of the carrier electrolyte as described in Section 2; b = separation with the same carrier electrolyte solution and sample as in (a) except that the solution in the electrode vessel at the injection valve was not protected from contact with ambient CO_2. For all of the conditions, see Fig. 5.

shifts for mononitrophenols (the weakest acids among the analytes) when the carrier electrolyte solution was not protected from contact with ambient CO_2. The selective shifts for mononitrophenols were linked with changes in their effective mobilities due to the decreased pH of the carrier electrolyte [24].

The separation efficiencies achieved for nitrophenols under our working conditions are summarized in Table 2. The use of a 300-μm I.D. capillary tube favouring enhanced sample loadability is less convenient as far as thermal dispersion is concerned. To make an estimate of its contribution to the overall dispersion, we employed a calculation procedure developed by Reijenga and Kenndler [30,31]. The results of these calculations for working conditions that were close to those used in our experiments revealed that the thermal effects could contribute 40–50% to the total value of the height of the theoretical plate.

3.4. Quantitative aspects

Concentration LODs for nitrophenols were estimated using a detection wavelength of 254 nm for the photometric detector and a 100-nl sample injection loop. The measurements were carried out in electrolyte system No. 3 at a driving current of 100 μA. The LOD data (Table 3) were calculated using a procedure developed for elution chromatography by Foley and Dorsey [32]. They clearly show the benefit of using a tube with an inner diameter of 300 μm compared with typical LOD values reported for 50–75 μm I.D. capillary tubes with on-column photometric absorbance detection [17,24,33,34]. The reproducibility of quantitating nitrophenols was assessed for low ppm concentrations (see Table 3). At concentrations corresponding to the limits of quantitation [35], the R.S.D. values were two–three times higher. The electropherogram shown in Fig. 7, obtained from the separation of a model sample containing nitrophenols at concentrations of 0.14–0.59 ppm, illustrates the limits of the elaborated CZE procedure in quantitation. The parameters of the calibration lines for concentrations of interest are given in Table 4.

3.5. Application potential

Nitrophenols often need to be determined in

Table 3
Limits of detection and reproducibilities in the determination of nitrophenols

Constituent	LOD (ppm	Concentration (ppm)	R.S.D.[a] (%)
o-NP	0.056	2.8	4.7
m-NP	0.027	2.8	5.4
p-NP	0.056	2.8	3.1
2,4-DNP	0.038	0.9	4.5
2,5-DNP	0.019	1.1	3.3
2,6-DNP	0.019	0.9	2.1
3,4-DNP	0.056	1.5	4.9
2,4,6-TNP	0.046	1.1	4.9
p-N-m-C	0.061	3.0	1.1
4,6-DN-o-C	0.080	5.9	1.6

[a] R.S.D. = relative standard deviation for three parallel determinations.

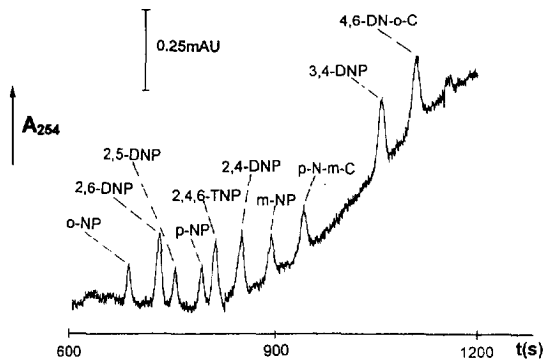

Fig. 7. CZE separation of nitrophenols present in a model sample at concentrations approaching their limits of quantitation. The concentrations of the analytes were, as follows: o-NP=0.4 ppm; 2,6-DNP=0.14 ppm; 2,5-DNP=0.17 ppm; p=NP=0.4 ppm; 2,4,6-TNP=0.34 ppm; 2,4-DNP=0.28 ppm; m-NP=0.17 ppm; p-N-m-C=0.46 ppm; 3,4-DNP=0.44 ppm; 4,6-DN-o-C=0.59 ppm. For further details, see Fig. 5.

various water matrices [1–10]. Therefore, rain, drinking and process water samples were chosen to test the improved procedure, especially, in terms of potentially disturbing matrix effects. These samples were spiked at two concentration levels. The first concentration level (0.45–2.0 ppm of the analytes) was intended for experiments with direct CZE analysis. The samples spiked with nitrophenols at a concentration of 70–120 ppb were used in CZE analysis combined with ITP sample pretreatment. Only electropherograms obtained from the experiments with drinking water are shown (Fig. 8 Fig. 9), as identical CZE profiles were obtained for all samples.

No interfering matrix effects were detected in the direct CZE analysis of the water samples. However, the LOD values attained under these conditions are too high to be of general practical applicability in the analysis of water samples [1–10]. Although an improvement in this respect can be expected by increasing the sample injection volume, there are inherent limits in using such an approach [36]. Sample preparation techniques applicable for nitrophenols [1,2,6–10] offer more convenient solutions in such situations. For CZE, the use of ITP pretreatment offers some favourable features. These include high recoveries, clean fractions of the analytes and

Table 4
Regression equations of calibration lines ($y=a+bx$) for nitrophenols

Constituent	a (mV s)	b (mV s/ppm)	r^a	Δx^b (ppm)
o-NP	$-1.87\cdot10^2$	$2.195\cdot10^3$	0.9944	0.40–6.0
m-NP	$1.87\cdot10^2$	$7.699\cdot10^3$	0.9976	0.17–2.8
p-NP	$0.02\cdot10^2$	$2.606\cdot10^3$	0.9995	0.40–7.0
2,4-DNP	$-0.70\cdot10^2$	$4.878\cdot10^3$	0.9993	0.30–5.0
2,5-DNP	$-1.18\cdot10^2$	$5.988\cdot10^3$	0.9996	0.17–2.8
2,6-DNP	$3.71\cdot10^2$	$7.457\cdot10^3$	0.9986	0.15–2.8
3,4-DNP	$1.11\cdot10^2$	$5.372\cdot10^3$	0.9998	0.45–7.4
p-N-m-C	$1.99\cdot10^2$	$2.995\cdot10^3$	0.9996	0.45–7.7
4,6-DN-o-C	$-1.83\cdot10^2$	$4.826\cdot10^3$	0.9997	0.60–9.9

[a] r=correlation coefficient.
[b] Δx=concentration intervals for which the calibration data were measured.

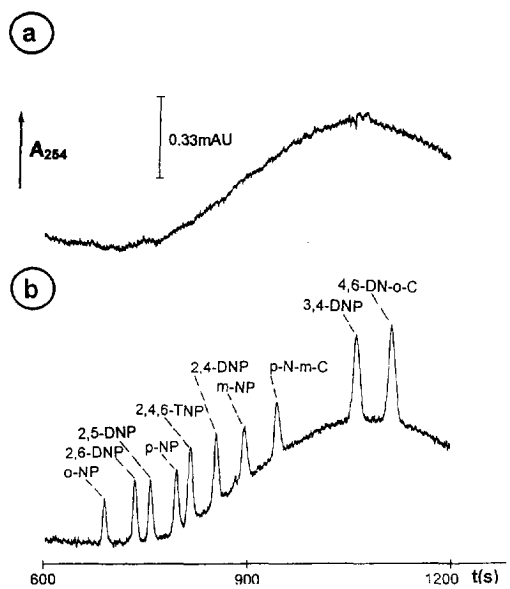

Fig. 8. CZE separation of nitrophenols present in a drinking water sample. a=drinking water sample (see Section 2); b=the same sample as in (a) but spiked with the nitrophenols that were being investigated. The concentrations of nitrophenols were: o-NP=1.4 ppm; 2,6-DNP=0.45 ppm; 2,5-DNP=0.55 ppm; p-NP=1.4 ppm; 2,4,6-TNP=1.15 ppm; 2,4-DNP=0.90 ppm; m-NP=0.55 ppm; p-N-m-C=1.55 ppm; 3,4-DNP=1.45 ppm; 4,6-DN-o-C=2.0 ppm. For further details, see Fig. 5.

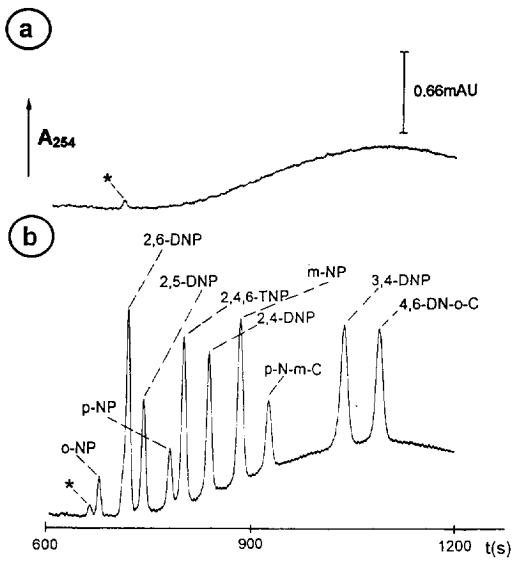

Fig. 9. CZE separation of the nitrophenols present in a drinking water sample following ITP pretreatment. a = Drinking water sample (a fraction from the ITP pretreatment in which nitrophenols are to be expected); b = the same sample as in (a) except that the sample was spiked with nitrophenols at the following concentrations: o-NP = 70 ppb; 2,6-DNP = 92 ppb; 2,5-DNP = 92 ppb; p-NP = 70 ppb; 2,4,6-TNP = 115 ppb; 2,4-DNP = 92 ppb; m-NP = 70 ppb; p-N-m-C = 75 ppb; 3,4-DNP = 92 ppb; 4,6-DN-o-C = 100 ppb before the pretreatment. Volumes (205 μl) of both samples were taken for ITP pretreatment. The ITP separation was carried out in electrolyte system ITP (Table 1). The fractions trapped by ITP for final CZE analyses were present in 20 μl volumes. For further details on the ITP pretreatment, see the text. CZE separations were carried out using the conditions described in the legend to Fig. 5.

well-defined separating conditions in the pretreatment, which can be considered to be orthogonal to the final CZE separation [22]. The electropherograms shown in Fig. 9 illustrate some of these advantages. From the point of view of detecting nitrophenols, they indicate that a further improvement could be expected if the limitations associated with the CZE injection technique used in this work could be overcome (of a 22–25 μl fraction containing nitrophenols after ITP pretreatment, only ca.0.5% could be accommodated by the CZE equipment). Steps to be taken to eliminate these limitations were discussed in detail elsewhere [22].

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References

[1] R. Herterich, J. Chromatogr. 549 (1991) 313.
[2] J. Feltes, in G. Angeletti and A. Bjoerseth (Editors), Organic Micropollutants in the Aquatic Environment, Kluwer, Dordrecht, 1991, p. 321.
[3] G.P. Bespamyatnov and Yu.A. Krutov, Predelno Dopustimye Koncentracii Khimicheskikh Vestchestv v Okruzhayushchei Srede, Khimiya, Leningrad, 1985.
[4] H.E. Wise Jr., P.D. Fahrenthold, Environ. Sci. Technol. 15 (1981) 1292.
[5] K. Nojiama, K. Fukaya, F. Fukui, S. Kanno, S. Nishiyama, Y. Wada, Chemosphere 1 (1976) 25.
[6] R.T. Coutts, E.E. Hargesheimer, F.M. Passutto, J. Chromatogr. 195 (1980) 105.
[7] K. Nick, H.F. Schoeler, Fresenius' J. Anal. Chem. 343 (1992) 304.
[8] H. Roseboom, C.J. Berkhoff, J.U. Wammes, R.C.C. Wegman, J. Chromatogr. 208 (1981) 331.
[9] B. Schultz, J. Chromatogr. 269 (1983) 208.
[10] H.B. Boehm, J. Feltes, D. Volmer, K. Levsen, J. Chromatogr. 478 (1989) 399.
[11] M.K. Sahoo, J. Kuruc, A. Švec, R. Čech, M. Hutta, J. Radioanal. Nucl. Chem. 163 (1992) 107.
[12] C.W. Klampfl, E. Spanos, J. Chromatogr. A 715 (1995) 213.
[13] S. Čechová, P. Rajec, J. Radioanal. Nucl. Chem. 101 (1986) 17.
[14] D. Kaniansky, V. Madajová, J. Marák, E. Šimuničová, I. Zelenský, V. Zelenská, J. Chromatogr. 390 (1987) 51.
[15] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, J. Chromatogr. 332 (1985) 211.
[16] C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee, S.F.Z. Li, J. Chromatogr. 516 (1990) 263.
[17] D. Martinez, E. Pocurull, R.M. Marcé, F. Borrull, M. Calull, J. Chromatogr. A 734 (1996) 367.
[18] D. Kaniansky, M. Masár, J. Marák, V. Madajová, F.I. Onuska, J. Radioanal. Nucl. Chem. 208 (1996) 343.
[19] E. Krčmová, M.Sc. Thesis, Comenius University, Bratislava, 1996.
[20] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, J.I. Akiyama, J. Chromatogr. 271 (1987) 51.
[21] M. Hutta, D. Kaniansky, E. Kovalčíková, J. Marák, M. Chalányová, V. Madajová, E. Šimuničová, J. Chromatogr. A 689 (1995) 123.

[22] D. Kaniansky, E. Krčmová, V. Madajová, M. Masár, Electrophoresis 18 (1997) 260.
[23] F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, Isotachophoresis: Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[24] F. Foret, L. Křivánková and P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[25] P. Molyneux, H.P. Frank, J. Am. Chem. Soc. 83 (1961) 3169.
[26] P. Molyneux, H.P. Frank, J. Am. Chem. Soc. 83 (1961) 3175.
[27] P. Molyneux, S. Vekavakayanondha, J. Chem. Soc. Faraday Trans. I 82 (1986) 291.
[28] P. Molyneux, S. Vekavakayanondha, J. Chem. Soc. Faraday Trans. I 82 (1986) 635.
[29] P. Moluneux, in G.A. Stahl and D.N. Schultz (Editors), Water-Soluble Polymers for Petroleum Recovery, Plenum, New York, 1988, p. 195.
[30] J.C. Reijenga, E. Kenndler, J. Chromatogr. A 659 (1994) 403.
[31] J.C. Reijenga, E. Kenndler, J. Chromatogr. A 659 (1994) 417.
[32] J.P. Foley, J.G. Dorsey, Chromatographia 18 (1984) 503.
[33] S.F.Y. Li, Capillary Electrophoresis—Principles, Practice and Applications, Elsevier, Amsterdam, 1992.
[34] S.L. Pentoney, Jr. and J.W. Sweedler, in J.P. Landers (Editor), Handbook of Capillary Electrophoresis, CRC, Boca Raton, FL, 1994, p. 147.
[35] D.L. Massart, B.G.M. Vandegiste, S.N. Deming, Y. Michotte and L. Kaufman, Chemometrics: A Textbook, Elsevier, Amsterdam, 1988.
[36] Th.P.E.M. Verheggen, A.C. Schoots, F.M. Everaerts, J. Chromatogr. 503 (1990) 245.