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Direct determination of phosphate esters in concentrated nitrate media by capillary zone electrophoresis

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Abstract

The potential of capillary zone electrophoresis (CZE) for the determination of dibutyl phosphate (DBP) and monobutyl phosphate (MBP), two degradation products of the tributyl phosphate extractant used in the nuclear fuel reprocessing industry, was evaluated. Analysis conditions were optimised, taking particularly into account that many determinations had to be performed in concentrated aqueous nitrate or nitric acid solutions. Separations were therefore carried out using the counter-electroosmotic mode with cathodic detection in a pH 8.3 electrolyte containing a suitably selected chromophore, salicylate, to ensure the indirect UV detection of the analytes. Various aspects of the method, including its sensitivity, working range, repeatability, and rapidity, were examined. Quantification of both phosphate esters was achieved in less than 3 min at concentrations ranging from 2×10^{-6} to 10^{-3} mol l^{-1} in samples containing no macro-component. The lower end of this range increased to 5×10^{-6} mol l^{-1} for MBP and 1.5×10^{-5} mol l^{-1} for DBP in samples containing 5×10^{-2} mol l^{-1} of sodium nitrate, thus enabling their determination in solutions containing nitrate or nitric acid at concentrations up to, respectively, 10 000 and 3000 times higher than the target analyte concentration. This simple, fast and reliable method is routinely applicable to aqueous samples with no other preliminary treatment than a proper dilution; analysis was also performed in organic matrices after a prior extraction. The method was validated by an excellent correlation with the standard DBP analysis technique, gas chromatography (GC). In order to develop appropriate chemical treatments to destroy these compounds, the method was applied to the monitoring of DBP and MBP degradation by hydrogen peroxide in 1 mol l^{-1} nitric acid solutions.

Keywords: Dibutyl phosphate; Monobutyl phosphate; Phosphate esters

1. Introduction

The most widely implemented method for reprocessing spent nuclear fuel uses liquid–liquid extractions (LLEs) to separate uranium and plutonium from the other elements formed in the reactor [1]. The extractant is a phosphoric ester, tributyl phosphate (TBP)

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($(C_4H_9O)_3P{=}O$). TBP, diluted in an organic medium, is brought into contact with nuclear fuel dissolution solutions in highly concentrated nitric acid. Under these conditions, TBP undergoes degradation by both radiolysis and hydrolysis, forming mainly dibutyl phosphate (DBP) ($(C_4H_9O)_2P{=}O(OH)$), and, to a lesser extend, monobutyl phosphate (MBP) ($(C_4H_9O)P{=}O(OH)_2$), phosphoric acid, and butanol [1,2], which diminish the reprocessing performance. It is therefore indispensable to be able to determine these products, particularly in concentrated nitric acid media, and to develop processes for eliminating them.

A few methods, principally chromatographic techniques, are currently available for analysing DBP and MBP (Table 1). Gas chromatography (GC) is the most widely used technique for the determination of DBP, mainly in the organic phase. Derivatisation is then necessary prior to analysis to increase the volatility of the compounds, but this preliminary step is time-consuming and may rise problems of incomplete reaction or by-products formation [1,3,4]. The methylated compounds can then be determined at concentrations ranging from 5×10^{-5} to 5×10^{-3} mol l^{-1} using a flame ionisation detector (FID) [5]. Liquid chromatography (LC) techniques use the anionic properties of DBP and MBP in basic media for determination by ion-exchange or ion-pair chromatography. This is the only MBP analysis method in use at the present time. Separation on anion exchange resins followed by conductivity measurement enables the determination of DBP and MBP in the range 8×10^{-7} to 5×10^{-6} mol l^{-1} and 2×10^{-7} to 4×10^{-6} mol l^{-1}, respectively, in aqueous media containing 1.6×10^{-3} mol l^{-1} nitrate [6] and analytes could also be detected in aqueous reprocessing solutions [7]. Separation by ion-pair chromatography is performed on an octadecyl silica stationary phase, using tetrahexylammonium bromide as an ion pairing agent. Prior concentration and purification by on-line SPE is necessary to determine traces amounts of DBP in aqueous solutions generated by reprocessing spent nuclear fuel; DBP can then be detected by refractometry at concentrations ranging from 2×10^{-5} to 5×10^{-4} mol l^{-1} [8]. The same method was applied to DBP and MBP analysis at concentrations of 10^{-3} mol l^{-1} in waste solutions containing nitrite and nitrate ions (1 mol l^{-1}) [9]. A precolumn purification is indispensable with such media to avoid masking the MBP peak by a large negative peak due to the presence of nitrate ions. An electrospray–mass spectrometry method has recently been developed to analyse DBP in organic media (containing TBP) and MBP in nitric acid media [10]. This technique does not require prior separation or derivatisation and provides satisfactory sensitivity for the determination of these compounds, with an MBP detection limit of 6×10^{-4} mol l^{-1} in a medium containing 3 mol l^{-1} nitric acid and 2 mol l^{-1} sodium nitrate; however, it requires sophisticated and costly equipment. Infrared spectrometry has also been employed to determine DBP in organic media, based on the P=O absorption band at 1230 cm^{-1}, after extraction with carbon sulfide. Nevertheless, this method is not only long and tedious, but also provides limited sensitivity [8]; moreover, simultaneous determination of MBP and DBP requires prior separation because of their similar spectra [1]. Other methods, including isotachophoresis [2,11] and potentiometry [12,13], have also been developed, but they are in very limited use. Acid–base titration methods are non-selective, and prior liquid–liquid extraction steps are required for nitric acid matrices [13]. An alternative routine method was therefore sought to separate and detect not only DBP but also MBP in aqueous phases with possibly high salt concentrations.

Capillary electrophoresis has emerged over the last decade as an increasingly high-performance separation technique that supplements conventional HPLC and ion chromatography methods [14,15]. Capillary electrophoresis combines the advantages of quick separation, high resolution, simple experimental implementation, low cost, small sample volume needed for analysis, and negligible consumption of electrolyte and organic solvent [16,17]. It is also a suitable alternative to traditional chromatography techniques for ion analysis in media containing large concentrations of other species, in which matrix effects could be a problem [14,18,19]. Despite these performances, few applications of capillary electrophoresis to the nuclear field have been proposed at present [20–22]. Capillary zone electrophoresis (CZE) however seemed to be ideally suited to the direct analysis of MBP and DBP in anionic form in aqueous media.

The aim of this work was to develop a simple CZE analysis method for the main TBP degradation products (MBP and DBP). The operating conditions were optimised, particularly the electrolyte composition, in

Table 1
Analytical methods described in the literature for the analysis of MBP and DBP

Method	Compound	Matrices	Sample pretreatment	Analysis conditions	Working range ($mol\,l^{-1}$)	LOD[a] ($mol\,l^{-1}$)	Repeatability, confidence interval	Analysis time (min)	Reference
GC	MBP, DBP	Organic media (TBP–alkane mixture)	Derivatisation (methylation)	FID detection	5×10^{-5} to 5.10^{-3}	10^{-6}	2%		[5]
					10^{-4} to 8×10^{-4}	5×10^{-5}	2×10^{-5}		[3,4]
		Aqueous media	LLE (NaOH) + derivatisation						[1]
Ion pair HPLC	DBP	Aqueous reprocessing solutions (cations, nitric acid, ...)	Acidification + on-line SPE on C_{18} silica (1 ml sample)	C_{18} silica, refractometric detection	2×10^{-5} to 5×10^{-4}	5×10^{-7}	1 to 2×10^{-7} M	15	[8]
		Organic media (dilute TBP)	LLE (NaOH) + as above					60	[8]
	MBP, DBP	1 M nitrate	On-line SPE on C_{18} silica (20 μl sample)	As above	1×10^{-3} to 5×10^{-2}	5×10^{-4}		20	[9]
Ion chromatography	MBP	Aqueous media 1.6×10^{-3} M nitrate	None (50 μl sample)	Anion exchanger, conductometric detection	2×10^{-7} to 4×10^{-6}	10^{-7}	5–7%	16	[6]
	DBP				8×10^{-7} to 5×10^{-6}	7×10^{-7}			
	DBP	Aqueous reprocessing solutions		As above	10^{-5} to 2×10^{-3}		0.2–6%	30	[7]
ESI-MS	MBP	Nitric acid	1/5000 dilution	Negative ionisation mode	6×10^{-4} to 6×10^{-2}	6×10^{-4}	2%		[10]
	DBP	TBP mixture	1/10 000 dilution	Negative ionisation mode	5×10^{-3} to 5×10^{-2}	2.5×10^{-5}	1%		[10]
				Positive ionisation mode	5×10^{-5} to 5×10^{-4}		2%		
Infrared spectrometry	MBP, DBP		Extraction by carbon sulfur; separation required	$1230\,cm^{-1}$		8×10^{-4}			[1]
Isotachophoresis	MBP, DBP	Irradiated TBP-aqueous solutions	None			5×10^{-6}		10	[2,11]
Potentiometry	MBP, DBP	Irradiated TBP-nitric acid solutions	LLE washings	Titration with NaOH		10^{-3}	5–6%		[12,13]

[a] LOD: Limit of detection.

which the chromophore added to detect analytes by indirect UV absorption, was carefully selected. Separation was also developed to analyse these compounds in matrices with high nitric acid or sodium nitrate concentrations. Various aspects of the method, including its sensitivity, working range, repeatability, and rapidity, were examined. In the context of a research program to identify reagents capable of destroying TBP degradation products, the method has been applied to monitor DBP and MBP degradation by hydrogen peroxide in nitric acid solutions.

2. Experimental

2.1. Instrumentation

A ThermoQuest SpectraPhoresis Ultra instrument (ThermoQuest, San Jose, CA, USA) equipped with a SpectraPhoresis UV3000 absorbance detector was used for capillary electrophoresis experiments. Separations were performed through untreated fused-silica capillaries of 40.5 cm × 50 μm i.d. from ThermoQuest. The length to the detection window was 34.5 cm.

Gas chromatography analyses were carried out on an Autosystem Gas Chromatograph (Perkin-Elmer, Las Vegas, NV, USA) equipped with a split–splitless injector and a flame ionisation detector. A 25 m × 0.25 mm i.d. CP-SIL 5 CB capillary column from Chrompack (Varian, Walnut Creek, CA, USA) coated with a 0.12 μm dimethylpolysiloxane film was employed.

2.2. Chemicals

Electrolytes were prepared using a LC-quality water obtained by purifying demineralised water in a MilliQ filtration system (Millipore, Milford, MA, USA). All chemicals used for the electrolytes were of analytical-reagent grade. *p*-Hydroxybenzoic acid (*p*HB), *o*-phtalic acid, pyrazine-2,3-dicarboxylic acid, pyridine-2,6-dicarboxylic acid, terephtalic acid, *tris*(hydroxymethyl) aminomethane (TRIS), sodium nitrate and dibutyl phosphate were purchased from Sigma–Aldrich–Fluka (Saint Quentin Fallavier, France). Tributyl phosphate, salicylic acid and methanol were supplied by Prolabo (Fontenay sous Bois, France). Monobutyl phosphate was purified from a commercialised mixture of DBP and MBP (Serlabo, Paris, France) by liquid–liquid extraction with a water/chloroform mixture. The concentration of MBP in the aqueous phase was evaluated at 5.3×10^{-2} mol l^{-1} by potentiometry.

2.3. Procedures

Standard solutions containing DBP and MBP at concentrations ranging from 10^{-6} to 10^{-3} mol l^{-1} were prepared from stock solutions of 0.1 mol l^{-1} DBP in a 50:50 (v/v) water/methanol mixture and 5.3×10^{-2} mol l^{-1} MBP in water.

For CZE experiments, the 1 ml samples analysed by CZE were prepared by mixing 500 μl of unknown sample (or standard solution) with 500 μl of electrolyte. Samples were injected using the hydrodynamic mode with a 0.8 psi pressure applied for 5 s, which corresponds to a 9.6 nl injection volume. A constant voltage of +30 kV was applied for the separation. The temperature was set to 20 °C. After optimisation, the electrolyte contained 10^{-2} mol l^{-1} salicylic acid adjusted to pH 8.3 with 2.9×10^{-2} mol l^{-1} TRIS. Analytes were monitored by indirect UV detection at 230 nm. Methanol was used as the electroosmotic flow marker.

New capillaries were conditioned by flushing successively with water at 30 °C for 1 min, at 60 °C for 1 min, then with 1 mol l^{-1} sodium hydroxide at 60 °C for 1 min and finally with water at 30 °C for 1 min. Between each run, the capillary was rinsed with the electrolyte for 1 min at a 100 psi pressure, which corresponds to the displacement of 20 capillary volumes. Each analysis was repeated three or four times.

For GC analyses, an internal standard (diisobutyl phosphate) was added to the samples. A 1 μl sample volume was injected into the column. The injector, set at 250 °C, was used in the split mode. Helium was employed as carrier gas at a 7 psi pressure. Separations were performed at 350 °C. The FID temperature was set to 300 °C. Each analysis was repeated three times.

Samples corresponding to the sonolytic degradation of TBP were analysed by CZE and GC. They were prepared as follows. Pure TBP solutions were submitted to ultrasounds (acoustic frequency of 20 kHz, acoustic intensity measured in water of 3.4 W cm^{-2}) for 3 h (sample K3), 6 h (sample K6) or 8 h (samples A8, B8,

C8 and K8). Acidic compounds formed by hydrolysis of TBP (among which DBP and MBP) were then extracted from the organic phase using a 1 mol l^{-1} NaOH aqueous solution. After a proper dilution, these basic solutions were directly analysed by CZE. For GC analyses, a washing of these solutions with isooctane first enabled the elimination of the co-extracted TBP. The aqueous solutions were then acidified with concentrated nitric acid and the acidic compounds were extracted with chloroform. The analytes contained in the chloroform phase were derivatised with diazomethane to convert organic acids to their methyl esters. The methylated sample was injected in the GC system.

In order to study DBP degradation, samples initially containing 3.4×10^{-2} mol l^{-1} DBP in 1 mol l^{-1} nitric acid were oxidised by a Fenton reagent. Hydrogen peroxide and a transition metal were added to the solution. Samples were collected after reactions times of 24, 48, 72 and 95 h. The reaction was then stopped with the addition of 2 mol l^{-1} $NaNO_2$. Samples were diluted 100 times with water and 5 mol l^{-1} NaOH was added to basicify the sample to a final pH between 9 and 10.

2.4. CZE calculations

Peak areas corrected by migration times (A/t_m) were considered for quantification. Peak asymmetry was defined at 10% of the peak height. Efficiency was measured by the theoretical plate number N calculated using the relation $N = 5.54(t_m/w_{1/2})^2$, where t_m is the migration time and $w_{1/2}$ the peak width at half height measured on the electropherogram. Repeatability was estimated either by the standard deviation σ for n repetitions of the experiment or by the relative standard deviation, calculated as hundred times the ratio of the standard deviation σ to the mean.

The electrophoretic mobility m^i_{ep} of the i compound was calculated as the difference between the electroosmotic mobility, obtained from the electroosmotic time t_{eo} and the apparent mobility m^i_{app}, obtained from the migration time t^i_m, using the relation $m^i_{ep} = lL/V(1/t_{eo} - 1/t^i_m)$, where L represents the total length of the capillary, l the length to the detector window and V the applied voltage. The mobility at infinite dilution $m^{i\infty}_{ep}$ was then obtained from the empirical relation valid within the ionic strength range 10^{-3} to 10^{-1} mol l^{-1}: $m^i_{ep} = m^{i\infty}_{ep} \exp(-\omega(|z|I)^{1/2})$, where I is the ionic strength of the electrolyte, z the analyte charge and ω the constant equal to 0.5 for $z = 1$ and 0.77 for $z = 2$ [23].

3. Results and discussion

3.1. Optimising the analytical conditions

Small anions are generally separated in the co-electroosmotic mode with anodic detection using an electroosmotic flow reverser [14,24]. The electrophoretic migration of the ions then adds to the electroosmotic flow, resulting in rapid separations. Preliminary testing however showed that this mode was unsuitable for DBP and MBP analysis in the presence of concentrated nitrate ions, as the analyte peaks were masked by the nitrate peak. Another mode was therefore selected, namely the counter-electroosmotic mode with cathodic detection, in which a positive voltage is applied to the electrode at the injection end of the capillary. The anions are thus taken toward the detector by the electroosmotic flow, while their natural electrophoretic mobility opposes this movement. The absolute value of their own mobility must be lower than that of the electroosmotic flow for the compounds to be detected. This technique is directly applicable to MBP and DBP analysis in aqueous media containing nitrate ions. MBP and DBP anions have indeed a sufficiently low electrophoretic mobility that they are carried toward the detector by the electroosmotic flow, while the smaller nitrate ions, with a higher electrophoretic mobility (in absolute value) than the electroosmotic mobility, return to the injection vial.

A basic electrolyte with a pH of 8.3, imposed by means of a *tris*(hydroxymethyl) aminomethane cationic buffer, was chosen to ensure a sufficient electroosmotic flow. The analytes are then present in their anionic form, as pK_a values of DBP and MBP are 1.7, 1.7 and 6.8, respectively [12].

The target species do not absorb in the UV-Vis region. A chromophore was consequently added to the electrolyte to allow their indirect UV detection. In order to ensure optimum analyte detectability, the chromophore should carry a charge of the same sign as the analytes, possess a similar mobility and a high molar absorptivity. Various chromophores, whose

Table 2
Comparison of the electrolytes[a] investigated for the determination of MBP and DBP (experimental data, unless mentioned)

		4-Hydroxybenzoate (*p*HB)	Salicylate	*o*-Phtalate	Pyrazine-2,3-dicarboxylate	Pyridine-2,6-dicarboxylate	Terephtalate
Chromophore characteristics							
	Mobility at infinite dilution ($\times 0.10^{-5}$ cm^2 V^{-1} s^{-1})[b]	34	35.4	52.9/28.1	39.4	47.4	–[c]
	Detection wavelength (nm)	246	230	227	279	214	240
	Molar absorptivity (l mol^{-1} cm^{-1})	12200	6900	6500	6800	9200	12000
Analyte determination							
MBP	Peak asymmetry	2.37 (0.04)	1.77 (0.12)	0.68 (0.01)	0.67 (0.01)	0.67 (0.01)	0.67 (0.01)
	Time-corrected peak area (mAU min^{-1})	156 (20)	63 (1)	62 (1)	54 (12)	57 (20)	43 (0.5)
DBP	Peak asymmetry	1.05 (0.04)	0.96 (0.13)	0.98 (0.03)	0.99 (0.07)	0.87 (0.01)	0.91 (0.01)
	Time-corrected peak area (mAU min^{-1})	49 (5)	39 (1)	22 (0.5)	24 (3)	33 (2)	30 (0.5)

Characteristics of the chromophores, asymmetry and time-corrected peak area values for MBP and DBP, at 2×10^{-4} mol l^{-1}, in these electrolytes. The values in brackets indicate the standard deviation, for $n = 3$ analyses.

[a] The electrolytes consisted of 10^{-2} mol l^{-1} chromophore adjusted to pH 8.3 with TRIS (TRIS concentration below 5×10^{-2} mol l^{-1}).

[b] Literature data.

[c] (–) Not mentioned.

characteristics are listed in Table 2, were tested. Their electrophoretic mobility varies from 3×10^{-4} to 5×10^{-4} cm^2 V^{-1} s^{-1}, which corresponds to the expected mobility range of MBP. The selection criteria were the peak asymmetry and peak areas corrected by migration time, corresponding to the detectability. Whatever the tested chromophore, the DBP peak was almost symmetrical, whereas the MBP peak was more asymmetrical (Table 2). Chromophores such as terephthalate, pyridine-2,6-dicarboxylate, pyrazine-2,3-dicarboxylate and *o*-phtalate proved to be too mobile compared to MBP (asymmetry factor lower than one). Two chromophores could be used: *p*-hydroxybenzoate and salicylate. The latter exhibits less asymmetry, its mobility is closer to that of MBP, and it ensures adequate detectability. An electrolyte containing 10^{-2} mol l^{-1} of salicylic acid with 2.9×10^{-2} mol l^{-1} of TRIS at pH 8.3 was thus selected. Separation was carried out with a +30 kV voltage, at 20 °C, with indirect UV detection at 230 nm. Analytes were then detected by a decrease in absorbance as their zone moves in front of the detector, since the chromophore concentration is decreased in their zone. This resulted in negative peaks, which were reversed on the figures into positive peaks by an automatic data treatment.

3.2. CZE analysis performances

Fig. 1 shows the CZE analysis of DBP, MBP and butanoate, each at a concentration of 10^{-4} mol l^{-1}, under the selected conditions. The 1.2 min peak on the electropherogram corresponds to electroosmosis. MBP (peak at 2.40 min), with greater inherent mobility than DBP by virtue of its smaller size and double negative charge, migrates after DBP (peak at 1.65 min). Butanoate, with intermediate mobility between that of DBP and MBP, was used as an internal standard for quantification. Separation was obtained quickly, even if performed in the counter-electroosmotic mode, and the analysis time did not exceed 3 min.

The method was assessed for performance in terms of repeatability, linearity and detection limits. The separation quality was also evaluated, considering peak efficiency and asymmetry factor. The peaks were relatively symmetrical and efficiencies of about 200 000 plates m^{-1} were obtained at a concentration of 10^{-5} mol l^{-1} (Table 3). Both compounds were clearly resolved; the resolution remained higher than 4 even with the addition of the internal standard. The repeatability of migration times and of time-corrected peak areas (A/t_m), expressed as the relative standard deviation, was determined for quadruplicate analyses

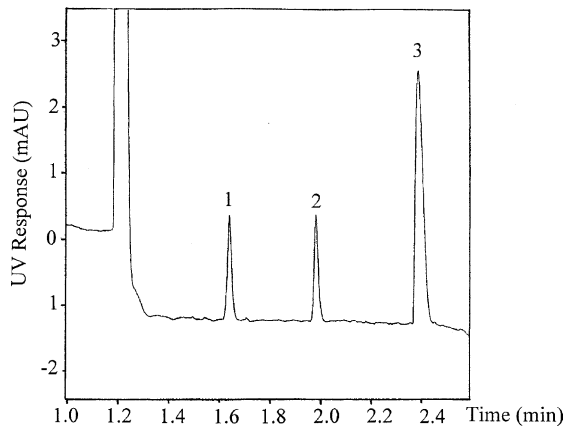

Fig. 1. CZE separation of a standard solution containing (1) DBP, (2) butanoate and (3) MBP, at 10^{-4} mol l^{-1} each. Experimental conditions: fused-silica capillary, 50 μm i.d. × 40.5 cm (34.5 cm to the detector window); hydrodynamic injection with a 0.8 psi pressure for 5 s; electrolyte, 10^{-2} mol l^{-1} salycilate + 2.9×10^{-2} mol l^{-1} TRIS (pH 8.3); separation voltage, +30 kV; temperature, 20 °C; indirect UV detection at 230 nm (the negative analyte peaks obtained by indirect detection were reversed by an automatic treatment).

at concentration levels ranging from 10^{-6} to 10^{-3} mol l^{-1}. The repeatability of migration times was assessed at about 2% within a series of 30 analyses and enables the proper identification of the compounds. The repeatability of time-corrected peak areas, indicated in Table 3, ranges from 1 to 5% for MBP concentrations above 5×10^{-6} mol l^{-1} and DBP concentrations above 5×10^{-5} mol l^{-1} and thus enables an accurate quantification. The dispersion increases as the concentration approaches the detection limit. Similar values are obtained whether quantified directly or with respect to the internal standard. The linearity domain of the A/t_m response versus the concentration was tested at concentrations ranging from 10^{-6} to 10^{-3} mol l^{-1} for MBP and 5×10^{-6} to 10^{-3} mol l^{-1} for DBP. It was linear with excellent correlation coefficients over the full test range, i.e. three orders of magnitude (Table 3). Comparable results were obtained with or without the internal standard. The detection limits, measured for a signal-to-noise ratio equal to three, were 1.5×10^{-6} and 2×10^{-6} mol l^{-1} for MBP and DBP, respectively, which compared well with the values routinely obtained for CZE using UV absorption [16,17].

The MBP and DBP electrophoretic mobilities were determined in these experimental conditions and proved to be constant at concentrations below

Table 3
Performances of the CZE analysis of MBP and DBP

	Concentration (mol l^{-1})	MBP	DBP
A/t_m repeatability (%)[a]	10^{-3}	4.9	4.5
	5×10^{-4}	0.9	0.5
	10^{-4}	3.8	3.8
	5×10^{-5}	1.3	4.4
	10^{-5}	4.4	16.5
	5×10^{-6}	5.3	15.9
	10^{-6}	12.8	21.7
Asymmetry	10^{-5}	1.35	1.21
Efficiency	10^{-5}	78500	55000
Linear regression data[a]		Direct	Direct
Slope (arbitrary units)		547	181
Intercept (arbitrary units)		0.6	0.5
Correlation coefficient (R^2)		1.0000	0.9999
Detection limit (mol l^1)		1.5×10^{-6}	2×10^{-6}

Precision for time-corrected peak areas (A/t_m), expressed as relative standard deviation ($n = 4$ repetitions), peak asymmetry, efficiency (theoretical plate number) with a 34.5 cm capillary (length to the detector window), linear regression data of time-corrected peak areas versus injection concentration and detection limits ($S/N = 3$). Operating conditions as in Fig. 2.

[a] Concentration ranging from 10^{-6} to 10^{-3} mol l^{-1} for MBP and 5×10^{-6} to 10^{-3} mol l^{-1} for DBP.

5×10^{-5} mol l^{-1}. The mobility at infinite dilution $m_{ep}^{i\infty}$ was then deduced using Friedl empirical equation [23] (see details in the Section 2), resulting in $m_{ep}^{i\infty}$ values of $(-17.5 \pm 0.1) \times 10^{-5}$ and $(-35.6 \pm 0.1) \times 10^{-5}$ cm^2 V^{-1} s^{-1} for DBP and MBP, respectively.

3.3. Effect of a high sodium nitrate concentration in the matrix

In many cases, MBP and DBP must be determined in solutions containing nitric acid or sodium nitrate at high concentrations compared with the target compounds. The CZE technique should be capable of analysing the target compounds in such matrices without prior SPE treatment; simply diluting the concentrated samples before analysis should be sufficient and would considerably shorten the sample preparation phase.

We therefore examined the effect of nitric acid or sodium nitrate in the sample matrix on CZE analysis of MBP and DBP. As the analysis must be performed in a basic medium, acidic samples were brought to a suitable pH (8–10) by addition of sodium hydroxide. Injecting a sample with a higher conductivity than that of the electrolyte will result in greater dispersion of the analyte zone and lessen detectability. The species in the high-conductivity zone actually will be subjected to a weak electric field and will migrate slowly; when they reach the boundary with the electrolyte zone, however, their velocity will be increased by the strong electric field in the less conductive electrolyte, so that the leading edge of the analyte zone will become broader. We determined the maximum sodium nitrate concentration compatible with acceptable detectability performance by testing sodium nitrate concentrations ranging from 2.5×10^{-2} to 10^{-1} mol l^{-1} in samples containing MBP, DBP and butyrate, each at 10^{-4} mol l^{-1}. The resulting electropherograms are shown in Fig. 2. A large peak corresponding to sodium ions appears before the electroosmotic peak. The chromophore concentration is actually increased in their zone, due to Kohlrausch regulation function [25], but the automatic data treatment reversed this positive peak into a negative one on the electropherograms. As for the nitrate ions, they returned to the vial located at the injection end of the capillary. The enrichment in nitrate ions in this vial, however, did not disturb the determination and over thirty successive analyses could be performed using the same electrolyte vial.

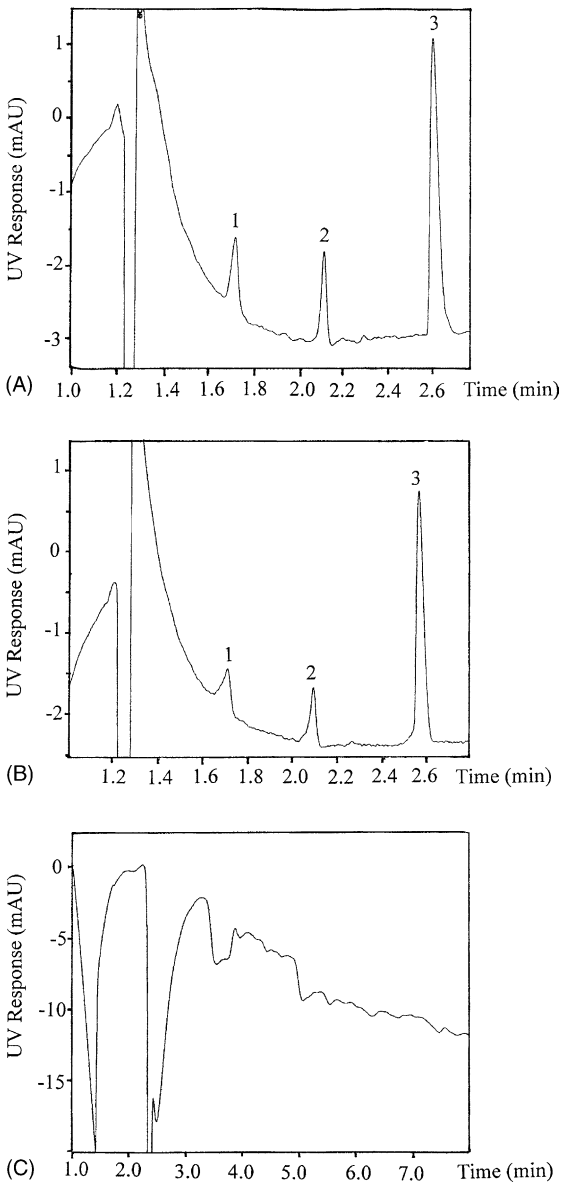

Fig. 2. Effect of $NaNO_3$ concentration in the sample matrix on CZE analysis of DBP and MBP: (A) 2.5×10^{-2} mol l^{-1}; (B) 5×10^{-2} mol l^{-1}; (C) 10^{-1} mol l^{-1} $NaNO_3$. Operating conditions as in Fig. 1.

High ion concentrations in the injected sample did indeed broaden the leading edge of the analyte peaks. In the matrices containing 10^{-1} mol l^{-1} of sodium nitrate, this effect was significant enough to prevent

Table 4
Performances of the CZE analysis of MBP and DBP in the presence of 5×10^{-2} mol l^{-1} sodium nitrate (for details see Table 3)

	Concentration (mol l^{-1})	MBP		DBP	
A/t_m repeatability (%)[a]	10^{-3}	3.0		5.4	
	5×10^{-4}	5.0		13.6	
	10^{-4}	3.6		4.8	
	5×10^{-5}	7.6		1.4	
	10^{-5}	14.4		29.3	
	5×10^{-6}	26.5		45.0	
	10^{-6}	41.2			
Efficiency	10^{-5}	42200		25900	
Linear regression data[a]		Direct	IS[b]	Direct	IS[b]
Slope (arbitrary units)		575	3.55	155	1.09
Intercept (arbitrary units)		−6	−0.01	−1	0.00
Correlation coefficient (R^2)		0.995	0.9997	0.989	0.9991
Detection limit (mol l^{-1})		5×10^{-6}		1.5×10^{-5}	

[a] Concentration ranging from 10^{-6} to 10^{-3} mol l^{-1} for MBP and 10^{-5} to 10^{-3} mol l^{-1} for DBP.
[b] IS: with an internal standard.

the detection of the analytes. At lower sodium nitrate concentrations (2.5×10^{-2} or 5×10^{-2} mol l^{-1}), the DBP peak was observed on the baseline retrace of the electroosmosis peak. However, as the objective was to maximize the sodium nitrate concentration with respect to the MBP and DBP concentrations, CZE performance was evaluated with samples containing 5×10^{-2} mol l^{-1} of sodium nitrate (Table 4).

The efficiency of the DBP and MBP peaks was then no more than half the efficiency obtained without sodium nitrate. The repeatability of the migration time was the same with or without sodium nitrate; MBP and DBP are therefore properly identified in highly concentrated matrices. The repeatability of time-corrected peak areas remained acceptable. Between 5×10^{-5} and 10^{-3} mol l^{-1}, the relative standard deviation was about 2–8% for MBP, which is less affected than DBP by the presence of sodium nitrate. The repeatability did not improve when the internal standard was used for quantification. The linearity of the calibration curves for MBP, drawn from 10^{-6} to 10^{-3} mol l^{-1}, and DBP, drawn from 10^{-5} to 10^{-3} mol l^{-1}, was poorer with sodium nitrate than without; using the internal standard improved the linearity, however, as shown by the correlation coefficients in Table 4, and will therefore be standard practice for actual sample analysis. The lower detectability with 5×10^{-2} mol l^{-1} sodium nitrate resulted in higher detection limits of 5×10^{-6} mol l^{-1} for MBP and 1.5×10^{-5} mol l^{-1} for DBP, for a signal-to-noise ratio equal to three.

The method can therefore be used to analyse DBP and MBP in solutions containing sodium nitrate at concentrations up to, respectively, 3000 and 10 000 times higher than the target analyte concentrations. These ratio can be reached for a sodium nitrate concentration in the sample of 5×10^{-2} mol l^{-1}. Samples containing a higher sodium nitrate concentration require a proper dilution before CZE analysis so as to bring it back to 5×10^{-2} mol l^{-1} and be able to determine MBP and DBP with the specified detection limits. These results, expressed as concentration ratio, are comparable with those obtained by ion chromatography, despite its greater sensitivity, as mentioned in Table 1 [6]. Using ion chromatography, trace levels of DBP and MBP could be detected in media containing nitrates at concentrations up to 2000 and 12 000 times higher, respectively. An advantage of CZE is the smaller sample volume and the shorter separation time, as the analysis lasts four times longer using ion chromatography [6].

3.4. Comparison with DBP analysis by gas chromatography

In order to validate the method, the results of DBP determination by CZE were compared with those obtained using the standard DBP analysis technique,

Table 5
Comparison of results obtained by CZE and GC. Concentration, in mol l^{-1}, of DBP and MBP (±standard deviation, $n = 3$) in sonolysed samples of TBP

Sample	GC	CZE	
	DBP	DBP	MBP
A8	2.38×10^{-4} ($\pm 0.02 \times 10^{-4}$)	2.27×10^{-4} ($\pm 0.15 \times 10^{-4}$)	8.49×10^{-4} ($\pm 1.1 \times 10^{-4}$)
B8	3.30×10^{-4} ($\pm 0.05 \times 10^{-4}$)	3.42×10^{-4} ($\pm 0.07 \times 10^{-4}$)	9.48×10^{-5} ($\pm 0.14 \times 10^{-5}$)
C8	6.40×10^{-4} ($\pm 0.08 \times 10^{-4}$)	6.21×10^{-4} ($\pm 0.10 \times 10^{-4}$)	1.19×10^{-4} ($\pm 0.02 \times 10^{-4}$)
K3	7.93×10^{-5} ($\pm 0.01 \times 10^{-5}$)	8.01×10^{-5} ($\pm 0.04 \times 10^{-5}$)	2.19×10^{-5} ($\pm 0.01 \times 10^{-5}$)
K6	1.87×10^{-4} ($\pm 0.003 \times 10^{-4}$)	1.89×10^{-4} ($\pm 0.01 \times 10^{-4}$)	4.44×10^{-5} ($\pm 0.07 \times 10^{-5}$)
K8	2.96×10^{-4} ($\pm 0.001 \times 10^{-4}$)	3.01×10^{-4} ($\pm 0.21 \times 10^{-4}$)	5.87×10^{-5} ($\pm 0.22 \times 10^{-5}$)

gas chromatography. DBP was analysed in various TBP organic solutions submitted to different ultrasonic treatments. Sonolysis of TBP actually formed both DBP and MBP.

GC analysis requires prior extraction of DBP and MBP in a basic aqueous medium to avoid interference by TBP during column separation. After a washing of the co-extracted TBP with isooctane, this aqueous solution is acidified and DBP and MBP are extracted again with chloroform, then methylated prior to analysis. For CZE analysis, only the first LLE step is necessary to transfer DBP and MBP to an aqueous phase. TBP, as a neutral compound, did not affect the CZE separation.

Table 5 shows an excellent agreement between both methods. When the DBP concentrations determined by CZE are plotted versus the GC determinations, the result is a straight line with a slope nearly equal to 1, a *Y*-intercept of practically zero ($y = 0.97x + 8 \times 10^{-6}$) and a correlation coefficient of 0.997. As expected, the measurement results are less scattered for the GC analysis (relative standard deviation between 0.1 and 1.5%) than for CZE (relative standard deviation between 1 and 8%). The analysis time is clearly in favour of CZE, since the CZE analysis lasted 3 min, plus 3 min for sample preparation, for a total of 6 min, whereas the GC analysis took 4 min, but also 15 min for methylation and 6 min for LLE, i.e. 25 min in all.

This comparison was established for samples in the organic phase. CZE is also simpler and faster for aqueous phase determinations, which are performed directly, whereas GC analysis requires an initial LLE in an organic medium followed by methylation before separation in the GC column.

3.5. Degradation of DBP and MBP by hydrogen peroxide in 1 mol l^{-1} nitric acid

As noted in Section 1, DBP and MBP can raise problems in the nuclear fuel reprocessing industry. Chemical methods have been tested to eliminate them

Table 6
CZE determination of MBP and DBP (± standard deviation, $n = 4$) in nitric acid samples oxidised by hydrogen peroxide

Quantification method; contact time (h)	Direct quantification		Standard addition	
	MBP (mol l^{-1})	DBP (mol l^{-1})	MBP (mol l^{-1})	DBP (mol l^{-1})
24	1.58×10^{-5} ($\pm 0.06 \times 10^{-5}$)	8.80×10^{-5} ($\pm 0.12 \times 10^{-5}$)	1.58×10^{-5} ($\pm 0.06 \times 10^{-5}$)	8.82×10^{-5} ($\pm 0.3 \times 10^{-5}$)
48	9.1×10^{-6} ($\pm 1.2 \times 10^{-6}$)	2.38×10^{-5} ($\pm 0.18 \times 10^{-5}$)	9.2×10^{-6} ($\pm 0.1 \times 10^{-6}$)	2.00×10^{-5} ($\pm 0.06 \times 10^{-5}$)
72	3.2×10^{-6} ($\pm 1.2 \times 10^{-6}$)	– [a]	2.4×10^{-6} ($\pm 0.2 \times 10^{-6}$)	– [a]
95	1.3×10^{-6} ($\pm 0.6 \times 10^{-6}$)	– [a]	2.3×10^{-7} ($\pm 0.5 \times 10^{-7}$)	– [a]

The values in the table correspond to the concentrations in the sample analysed by CZE (i.e. initial sample diluted 100 times).
[a] Not detected.

when they are present in concentrated nitric acid media. These include oxidation by hydrogen peroxide catalysed by a transition metal (Fenton's reagent [26]). DBP, initially at 3.4×10^{-2} mol l^{-1} in 1 mol l^{-1} nitric acid, was degraded by Fenton's reagent. It produced numerous compounds including MBP, which was in turn degraded. The destruction of DBP and the variation in the MBP concentration over time were monitored using the CZE method developed and validated above. After various exposure times, sodium nitrite and sodium hydroxide were successively added to the samples to stop the reaction and ensure a basic pH before analysis. Consequently, the final samples contained 3 mol l^{-1} sodium and 3 mol l^{-1} nitrate. For CZE determination, they were diluted to obtain target concentrations of 10^{-5} to 10^{-4} mol l^{-1}.

The analysis conditions had to be adapted. The first test indeed, conducted with a sample dilution factor of 50 at a voltage of +30 kV, highlighted that a compound identified as propanoate interfered with MBP and prevented its quantification (Fig. 3A). The separation was improved by adjusting the dilution factor and the applied voltage. Diluting the sample by a factor of 100 improved the resolution (Fig. 3B) but not enough; it was also necessary to reduce the voltage to +20 kV to obtain satisfactory separation (Fig. 3C). The analysis time thus increased to 4 min.

Butanoate was used as the internal standard. In each sample, we checked that none was present as a degradation product. DBP and MBP were identified by their migration time and by spiking the sample with standards. Two quantification methods were used: directly from the calibration curves previously established with 5×10^{-2} mol l^{-1} sodium nitrate, and by spiking the sample with known concentrations of DBP and MBP. In the second method, the desired concentration is the opposite of the *X*-intercept when the time-corrected peak area is plotted versus the spike concentration. An example of this method is shown in Fig. 4. The values obtained by both quantification methods, indicated in Table 6, coincide for the 24 and 48 h samples, but differ significantly for MBP in the 72 and 95 h samples, although this can easily be attributed to the low MBP concentrations, at or below the detection limit. In this case, the most reliable results were provided by the standard addition method.

Under the experimental conditions, the DBP disappeared completely after 72 h in contact with hydrogen peroxide. The MBP formed in the process was also gradually degraded and disappeared after 95 h of exposure to the reagent.

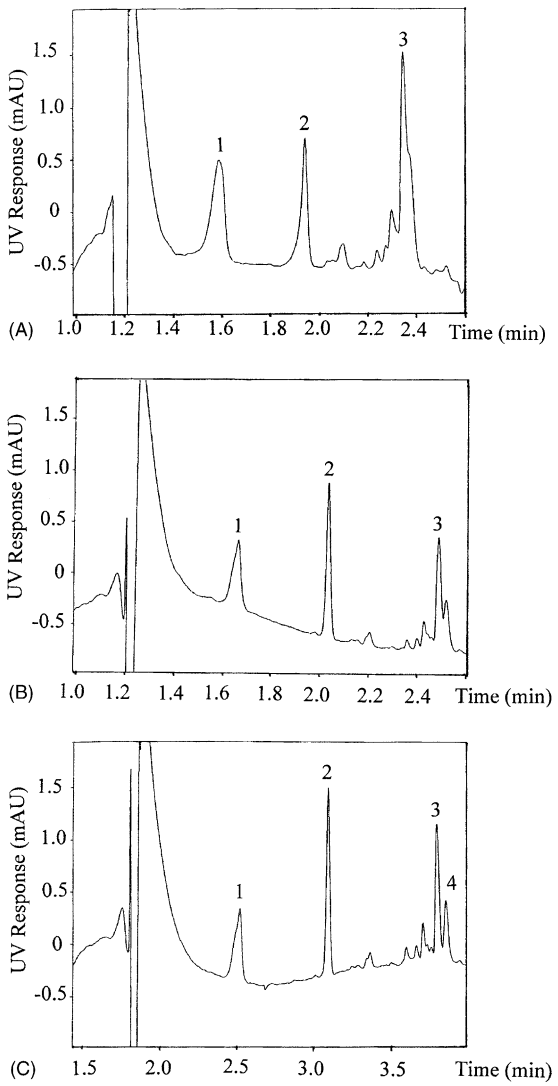

Fig. 3. Optimisation of CZE analysis of MBP and DBP in nitric acid samples degraded by hydrogen peroxide: (A) dilution factor 50, separation voltage +30 kV; (B) dilution factor 100, separation voltage +30 kV; (C) dilution factor 100, separation voltage +20 kV. (1) DBP; (2) butanoate; (3) MBP; (4) propanoate. Other conditions as in Fig. 1.

As shown by this example, capillary electrophoresis can be used to determine DBP and MBP in matrices containing a large number of elements, and notably sodium nitrate ions.

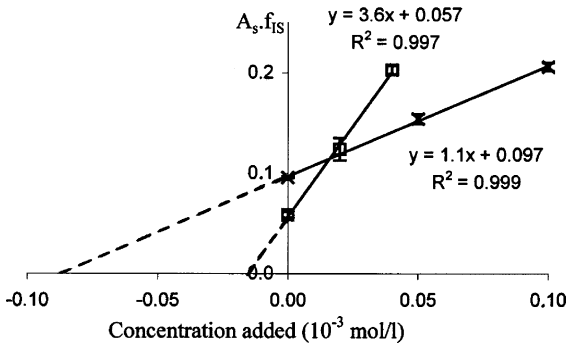

Fig. 4. Determination of MBP (□) and DBP (×) in samples degraded for 24 h by hydrogen peroxide using the standard addition method. Time-corrected peak area brought to the internal standard ($A_s f_{IS}$) versus the MBP or DBP concentration added to the sample.

4. Conclusions

Capillary zone electrophoresis proves to be ideally suited to the determination of DBP and especially MBP, for which few methods are currently available, in aqueous media. This technique, employed in the counter-electroosmotic mode with indirect UV detection at the cathode, also enables the investigation of MBP and DBP in the presence of nitrates or nitric acid at high concentrations. A feature of the experimental conditions developed in this work is the use of electroosmosis to avoid interference by nitrate ions in the sample matrix.

With a suitably selected chromophore—salicylic acid in this case—it is possible to determine MBP at concentrations ranging from 1.5×10^{-6} to 10^{-3} mol l^{-1} and DBP at concentrations ranging from 2×10^{-6} to 10^{-3} mol l^{-1}, without sodium nitrate. In samples containing 5×10^{-2} mol l^{-1} of sodium nitrate, the working range becomes 5×10^{-6} to 10^{-3} mol l^{-1} for MBP and 1.5×10^{-5} to 10^{-3} mol l^{-1} for DBP. The correlation coefficients are better than 0.999 for the calibration curves obtained using an internal standard (with or without sodium nitrate), and concentrations could be obtained with a 1–8% precision between 10^{-5} and 10^{-3} mol l^{-1}. The detection limits are relatively low (the lower limits of the above-mentioned concentration ranges) and the repeatable migration times ensure identification of the target compounds. The CZE method was validated by the very good correlation with the standard DBP determination technique, gas chromatography. CZE can therefore be used to analyse DBP and MBP in solutions containing sodium nitrate at concentrations up to 3000 and 10 000 times higher than the target analyte concentrations. Its application to the determination of DBP and MBP in samples containing large concentrations of sodium nitrate together with many other compounds required the optimisation of selected parameters (dilution factor and separation voltage) to enhance the resolution and improve the quantification accuracy. The same method was also employed for organic media containing TBP after a prior liquid–liquid extraction by a basic aqueous solution.

Capillary zone electrophoresis is thus a simple, high-performance method for routine use in analysing MBP and DBP in dilute aqueous media as well as in concentrated nitrate or nitric acid solutions or in organic media. It does not require prior sample treatment other than dilution in some cases (1:100 for 3 mol l^{-1} nitrate matrices). The performance obtained in terms of working range, sensitivity, repeatability, or analysis time is fully comparable with the results of the chromatographic methods listed in Table 1.

In addition to its technical performance, capillary electrophoresis is also cost-effective because of its short separation time (MBP and DBP analysed in less than 4 min), inexpensive consumables (fused-silica capillaries), negligible electrolyte consumption (a few millilitres a week) and extremely small sample volumes (about 10 nl per analysis). The last two points make this technique particularly attractive for investigating radioactive samples.

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