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Comparative study of aqueous and non-aqueous capillary electrophoresis in the separation of halogenated phenolic and bisphenolic compounds in water samples

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Abstract

Capillary zone electrophoresis methods, based on either aqueous and non-aqueous solutions as running buffers and UV spectrophotometric detection, have been developed and optimized for the separation of several halogenated phenolic and bisphenolic compounds, suspected or proved to exhibit hormonal disrupting effects. Both aqueous capillary electrophoresis (CE) and non-aqueous capillary electrophoresis (NACE) methods were suitable for the analysis of compounds under study. The separation of the analytes from other 25 potentially interfering phenolic derivatives was achieved with NACE method. Large-volume sample stacking using the electroosmotic flow pump (LVSEP) was assayed as on-column preconcentration technique for sensitivity enhancement. LVSEP–CE and LVSEP–NACE improved peak heights by 5–26 and 16–330 folds, respectively. To evaluate their applicability, the capillary electrophoresis methods developed were applied to the analysis of water samples, using solid-phase extraction as sample pre-treatment process.

Keywords: Brominated flame retardants; Bromophenols; TBBPA; TCBPA; Aqueous capillary electrophoresis; Non-aqueous capillary electrophoresis (NACE); Large-volume sample stacking

1. Introduction

Flame retardants are substances used in plastics, textiles, electronics circuitry and other materials to prevent fires. Some of the technical flame-retardant products contain brominated organic compounds that comprise an estimated 30% of the volume of all flame retardants employed [1].

Tetrabromobisphenol A (TBBPA) is a widely used brominated flame retardant [2]. Pentabromophenol (PeBP), 2,4,6-tribromophenol (2,4,6-TriBP), 2,4-dibromophenol (2,4-DiBP) and tetrachlorobisphenol A (TCBPA) are also used as halogenated flame retardants [3]. The halogenated phenols 2-bromophenol (2-BP), 2,4-DiBP, 2,6-dibromophenol (2,6-DiBP) and 2,4,6-TriBP were products generated in the thermal decomposition of TBBPA and/or from plastics treated with a polybrominated epoxy type flame retardants [4,5]. 2,4,6-TriBP is the main breakdown product from the decomposition of TBBPA when it is exposed to UV light [6]. Also it has been observed the presence of methylmonobromophenols on technical mixtures of brominated flame retardants (BFRs) [7].

So far, knowledge on the toxicity of the halogenated flame retardants is limited to a few congeners. Concerning phenolic compounds with one or two hydroxyl groups, due to structural similarities with hormones may play a key role as competitors of the natural hormones [8]. Compounds like TBBPA, 2,4,6-TriBP, PeBP, TCBPA and 2,4-DiBP have proved toxicity [9–13] and some of them may have comparable effects to the thyroid-disrupting effects of PCBs [11].

Halogenated phenolic compounds accumulate in the food chain and finally in humans, and are thus a potential environmental health problem. Not surprisingly, the levels of these compounds in the environment have increased as well [6,14], and thus, halogenated flame retardants levels in the environment must be monitored.

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Currently, several analytical methods are available for the determination of halogenated flame retardants in a variety of sample types [14]. By far, gas chromatography (GC) is the analytical technique of choice for the determination of this class of compounds [15–23]. TBBPA has been found in sewage sludge [15,16], sediments [15,17], indoor air [18], eggs from birds [19] and human serum [20]. TBBPA, 2,4,6-TriBP, PeBP, and TCBPA were determined in human plasma [21] and milk [22]. 2,4,6-TriBP also has been found in human serum [20], adipose tissue [23] and sewage sludge [16]. Also, liquid chromatography (HPLC–UV) has been used for the analysis of TBBPA and 2,4,6-TriBP from polymeric materials [14,24,25].

Capillary electrophoresis (CE) is a powerful technique of separation. The separation power in aqueous solution can be affected by various factors, like pH, nature of the buffering ion or organic modifiers in the electrolyte. The flexible adjustment of many chemical, physical and instrumental parameters allows stable performance to be achieved in aqueous CE. Nowadays, there is no reason to consider water as the only useful solvent in CE. Organic solvents have been used successfully in background electrolytes (BGE), extending the application range of the technique [26–30].

One of the major drawbacks of most widespread CE apparatus is the low sensitivity of conventional UV detection, limiting its application in trace analysis. To overcome this problem, samples can be concentrated directly on the capillary (on-column stacking). Large-volume sample stacking using the electroosmotic flow pump (LVSEP) has emerged as a highly efficient sample stacking method in aqueous media [31]. Chien and Burgi [32] demonstrated that large-volume hydrodynamic injection followed by the removal of the sample matrix out of the capillary using polarity switching is a powerful tool for sensitivity enhancement in CE. Later, this method was simplified by adding an electroosmotic flow (EOF) modifier to the running buffer [33], such as methanol as BGE solvent. Under a reverse electric field, the sample plug was removed from the capillary by itself and anionic analytes were separated without polarity switching [31].

In this paper, the application of CE as an alternative technique for the separation of several phenolic and bisphenolic compounds of environmental concern is shown. Aqueous and non-aqueous capillary electrophoresis (NACE) procedures have been developed and compared. A study of potential interferences has been carried out in order to assess the applicability of developed methods to environmental samples. Also, LVSEP was evaluated as on-column preconcentration technique allowing significant sensitivity enhancements.

2. Experimental

2.1. Reagents and materials

Methanol (HPLC gradient grade) was obtained from Merck (Darmstadt, Germany) and dimethyl sulfoxide (DMSO) (HPLC gradient grade) from Aldrich (Madrid, Spain). 2-Bromophenol (2-BrP, 98%), 2,4-dibromophenol (2,4-DiBP, 95%), 2,4,6-tribromophenol (2,4,6-TriBP, 99%), pentabromophenol (PeBP, 96%), tetrabromobisphenol A (TBBPA, 97%), tetrachlorobisphenol A (TCBPA, 98%), 2-bromo-4-methylphenol (2-Br-4-MeP, 96%), 4-bromo-3-methylphenol (4-Br-3-MeP, 99%), phenol (99%), bisphenol A (BPA, 97%), 4-nonylphenol (4-NonylP, tech.), 3-chlorophenol (3-CP, 98%), 4-chlorophenol (4-CP, 99%), 2,3-dichlorophenol (2,3-DiCP, 98%), 2,5-dichlorophenol (2,5-DiCP, 98%), 2,6-dichlorophenol (2,6-DiCP, 99%), 3,4-dichlorophenol (3,4-DiCP, 99%), 3,5-dichlorophenol (3,5-DiCP, 97%), 2,3,5-trichlorophenol (2,3,5-TriCP, 99%), 2,4,5-trichlorophenol (2,4,5-TriCP, 99%), pentachlorophenol (PeCP, 99%), 2,4-dimethylphenol (2,4-DiMeP, 98%), 2-nitrophenol (2-NP, 99%), 4-nitrophenol (4-NP, 99%) and 2,4-dinitrophenol (2,4-DiNP, 97%) were obtained from Aldrich (Madrid, Spain). 2,6-Dibromophenol (2,6-DiBP, >97%) was from Fluka (Buchs, Switzerland). 2,3,4,5-Tetrachlorophenol (2,3,4,5-TetraCP, 99%) was purchased from Supelco (Bellefonte, PA, USA). 2,3,4-Trichlorophenol (2,3,4-TriCP, 99%), 2,3,6-trichlorophenol (2,3,6-TriCP, 99%), 2,4,6-trichlorophenol (2,4,6-TriCP, 99%), 2,3,4,6-tetrachlorophenol (2,3,4,6-TetraCP, 99%) and 2,3,5,6-tetrachlorophenol (2,3,5,6-TetraCP, 99%) were from Riedel-de Häen (Seelze, Germany). 2,4-Dichlorophenol (2,4-DiCP, 98%), 2-methyl-4,6-dinitrophenol (2-Me-4,6-DiNP, 98%), sodium tetraborate decahydrate (GR, 99.5%) and sodium hydroxide (pellets GR for analysis, 99%) were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system purchased from Millipore (Bedford, MA, USA).

Cellulose ester membrane filters (SMWP, 47 mm, 5 μm; HAWP, 47 mm, 0.45 μm), Durapore membrane filters (GVHP, 47 mm, 0.22 μm), and Durapore Millex syringe filters (SLHV, 25 mm, 0.45 μm) were supplied by Millipore (Bedford, MA, USA). Oasis SPE cartridge columns packed with polystyrene–divinylbenzene (PS–DVB) sorbent (HLB, 60 mg, 3 cm^3) were obtained from Waters (Milford, MA, USA).

2.2. Preparation of standard solutions

Stock solutions (4.0 mg/mL) of each phenol derivative were prepared in methanol. Standard's mixtures for calibrations were diluted in methanol to appropriate concentration levels. All solutions were refrigerated at 4 °C and protected against daylight. These solutions were used to make daily working standards solutions by appropriate dilution.

In the measurements of the electroosmotic flow, DMSO was used as EOF marker at a concentration of c.a. 10 μg/mL. This solution was either mixed with the analytes under study or directly diluted with methanol.

2.3. CE analysis

Capillary electrophoresis was performed using a HP^{3D} system (Hewlett–Packard, Waldbronn, Germany) equipped with a photodiode array detection (DAD) system. Absorbances at 210, 230 and 370 nm (direct UV detection) were monitored for the detection of the analytes, depending of species considered in each particular mixture.

Uncoated narrow-bore silica capillary (supplied by Composite Metal Services Ltd., Ilkley, UK) with an effective/total length of 61.5/70 cm and 75 μm I.D. was used. The capillary was thermostated to 25.0 °C, unless otherwise stated.

A Chrompack RTE-110B external water bath (Neslab Instruments Inc., Newington, NH, USA) was used for thermostating the samples to 25 °C.

Standards and samples were injected hydrodynamically by applying a pressure of 50 mbar. Depending on the experiment injection time ranged from 2 to 300 s. The applied voltage for separation was 30 kV, either positive or negative.

The migration order was determined by injecting the individual solution of each compound and by the spectral comparison of each peak in electropherograms with a spectral library.

Independently of the BGE solvent, new capillaries were rinsed with 1 M sodium hydroxide for 20 min. Before injections, capillaries were conditioned by washing them with 0.1 M sodium hydroxide for 5 min, Milli-Q water for 5 min, and 15 min with the separation electrolyte. After each run the capillary was flushed with the solvent corresponding to the electrophoretic medium for 5 min and with Milli-Q water for 5 min. The inlet and outlet of the capillary were kept overnight in Milli-Q water.

Water and methanol were assayed as solvents for BGE preparation. Sodium tetraborate, being readily soluble in both solvents, was used as electrolytic salt. The pH of the solution was adjusted by addition of a sodium hydroxide solution in the same BGE solvent. Measured pH values of buffer solutions were obtained with a Metrohm 654 pH-meter (Herisau, Switzerland) calibrated with aqueous standard buffer solutions. For practical reasons, in methanolic running buffers the apparent pH (pH_{app}) [34], instead of the thermodynamic pH, was considered more suitable to describe our systems. The electrolyte solution was prepared freshly every two days, sonicated in a P-Selecta ultrasonic bath (Barcelona, Spain) for at least 4 min and filtered through a membrane of 0.22 μm pore size. Every day all remaining solutions were filtered through a 0.45 μm syringe filter before use.

Data acquisition was done by means of HP^{3D} ChemStation Software (Rev. A.06.01[403]) (Hewlett–Packard, Waldbronn, Germany). Statistical analysis of the response variables was carried out using the statistical package Statgraphics Plus 3.3 (STSC, Rockville, MD, USA).

Oasis SPE cartridges were dried using a Turbo-Vap II Nitrogen Evaporator supplied by Zymark (Hopkinton, MA, USA).

2.4. LVSEP

All analytes were dissolved in methanol and introduced hydrodynamically into the capillary with a pressure of 50 mbar for different periods of time, depending on the experiment. In methanolic medium, and for those analytes that must be analysed in negative polarity, after sample injection a negative voltage of −30 kV was applied for both sample stacking and subsequent separation of analytes. However, in aqueous CE and in NACE separations carried out in positive polarity, after the stacking with negative voltage, the separation was carried out with polarity switching (+30 kV), when it was achieved about 95% of the corresponding buffer conductivity.

Fresh electrolyte and sample solutions were always used for each injection.

2.5. Sample preparation

An off-line solid-phase extraction (SPE) step was used to cleanup and preconcentrate the samples before CE analysis. Details of this preconcentration stage have been described elsewhere [35]. Real water samples were collected in a wastewater-treatment plant near Santiago de Compostela (North-West Spain).

Water samples, at their natural pH (pH 6.8–7), were filtered through a 0.45 μm cellulose ester membrane filters before the SPE (in some cases, for influent wastewater samples with high content of particulate matter, samples needed prefiltering through 5 μm membrane filters). The SPE Oasis cartridges were conditioned by passing 4 mL of methanol and 4 mL of Milli-Q water. After that, the water sample was loaded through the cartridge. Finally, the cartridge was washed with 10 mL of Milli-Q water, and then dried under nitrogen stream for 45 min at a pressure of 12 psi. The analytes trapped on the sorbent were eluted with 3 mL of methanol, and this extract was subsequently subjected to CE analysis.

3. Results and discussion

3.1. Optimization of electrophoretic separation by CE in aqueous and non-aqueous media

All of the compounds under investigation have weakly acid hydroxyl groups so capillary zone electrophoresis (CZE) at high or moderate pH might be suitable for their determination. The pK_a values in water of the considered bromophenols and halogenated bisphenols range from 4.4 to 9.5 which means that at pH values over 9.5 they are expected to be all at least partially dissociated (see Table 1), ready for CE analysis. Sodium tetraborate was chosen as electrolyte salt, either in aqueous and non-aqueous CE.

For every assayed electrolyte, it has been proved that electric field strength did not affect significantly the separation efficiency. As expected migration times decreased as the

Table 1
pK_a values in water of the compounds studied at $T = 25\,^{\circ}C$ (electroosmotic mobility (μ_{eo}) and effective mobilities (μ_{eff}) of the analytes in 20 mM sodium tetraborate solutions at optimum pH value)

Aqueous CE ⇒ BGE solvent: water			Non-aqueous CE ⇒ BGE solvent: methanol	
Compound	pK_a	μ_{eff} (10^{-4} cm^2 V^{-1} s^{-1}) pH 9.6	Compound	μ_{eff} (10^{-4} cm^2 V^{-1} s^{-1}) pH_{app} 9.4
4-Br-3-MeP	9.50[a]	−1.97	4-Br-3-MeP	−0.26
2-Br-4-MeP	8.73[a]	−2.67	2-Br-4-MeP	−0.34
PeBP	4.43[a]	−2.77	2-BrP	−0.55
2,4,6-TriBP[b]	6.10[c] 6.31[d]	−2.86	2,4-DiBP TCBPA	−2.18
2,4-DiBP	7.80[e]	−2.97	TBBPA	−2.24
2-BrP	8.44[e] 8.29[f]	−3.17	2,6-DiBP	−2.59
2,6-DiBP	6.60[e]	−3.22	PeBP	−2.67
TBBPA	7.50 (pK_{a1})[g] 8.50 (pK_{a2})[g]	−3.41	2,4,6-TriBP	−2.69
TCBPA	7.50 (pK_{a1})[h]	−3.51		
$\mu_{eo} = 6.68 \times 10^{-4}$ cm^2 V^{-1} s^{-1}			$\mu_{eo} = 1.27 \times 10^{-4}$ cm^2 V^{-1} s^{-1}	

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (© 1994 –2004 ACD/Labs), from [36].
[b] $pK_a^* = 10.10$ in methanol [37].
[c] From [37].
[d] From [38].
[e] From [39].
[f] From [40].
[g] From [3].
[h] From [41].

potential was increased. Then the highest allowable voltage (30 kV) was applied to speed up separations.

Capillary and sample tray temperatures were tested in the range of 18.5–30.0 °C in aqueous CE, and between 18.5 and 25.0 °C in NACE. In the remaining experiments, a temperature of 25 °C was selected as a good compromise between analysis time in aqueous CE and easy control of temperature in NACE.

3.1.1. Aqueous BGE

As electrophoretic separations are based on differential rates of migration of charged particles in the bulk of a running buffer, the effective mobility of weakly acid compounds is strongly affected by the pH of the electrolyte solution, as it determines the extent of ionization of the analytes [42].

The dependence of the mobility of the compounds studied with the pH was evaluated by calculating the effective electrophoretic mobilities (μ_{eff}) of the analytes working with 20 mM aqueous sodium tetraborate solutions at different pH values. According to results, the studied analytes may be grouped into two classes. The first group includes the compounds exhibiting higher pK_a values (4-Br-3-MeP, 2-Br-4-MeP and 2-BrP). As expected, this group appears heavily affected by changes in the electrolyte pH value in the range considered (9.2–9.8). On the other side, the remaining compounds are fully deprotonated in the pH range considered, so their effective mobilities are hardly affected.

At pH 9.2, some of the compounds co-migrated, such as 2,4-DiBP and 2-BrP, while others like PeBP and 2,4,6-TriBP were not fully separated. Electrolyte solutions at pH 9.4 and 9.6 provided enhanced resolution of all peaks, and pH 9.6 leads to a fully satisfactory separation (see Fig. 1A). However, increasing pH buffer to 9.8, worsen the separation, because some analytes (2-Br-4-MeP and PeBP, in addition to 2-BrP and 2,6-DiBP) co-migrate appreciably.

Under the finally optimised conditions, the electroosmotic mobility (μ_{eo}) was higher (in absolute value) than the electrophoretic mobilities (μ_{eff}) of the analytes (see Table 1).

The selection of buffer concentration depends also on separation requirements: high ionic strengths must be used for closely related analytes [43], because the double layer compression causes a reduction in EOF, increasing the migration times of anions in positive polarity mode, and therefore, leading to slow separations.

Three concentration levels of the running buffer were assayed: 20, 40 and 60 mM. The results indicated that although a slightly better resolution can be attained on increasing electrolyte concentration from 20 to 60 mM, this change produced an increase of about twice in the migration time of the analytes thus increasing the total analysis time unacceptably.

3.1.2. Non-aqueous BGE

Methanol is the most commonly used organic solvent in CE [44]. It has favourable properties, such as dielectric constant, viscosity, and a useful UV range for detection [45]. It is an appropriate solvent for common electrolyte salts, and it allows LVSEP as field-amplified technique for on-line concentration in NACE (see the next section). Moreover, is a solvent used as extractant in many sample pretreatment processes, like SPE, allowing the direct injection of the extracts in the NACE system. For these reasons, it was chosen as the running buffer solvent.

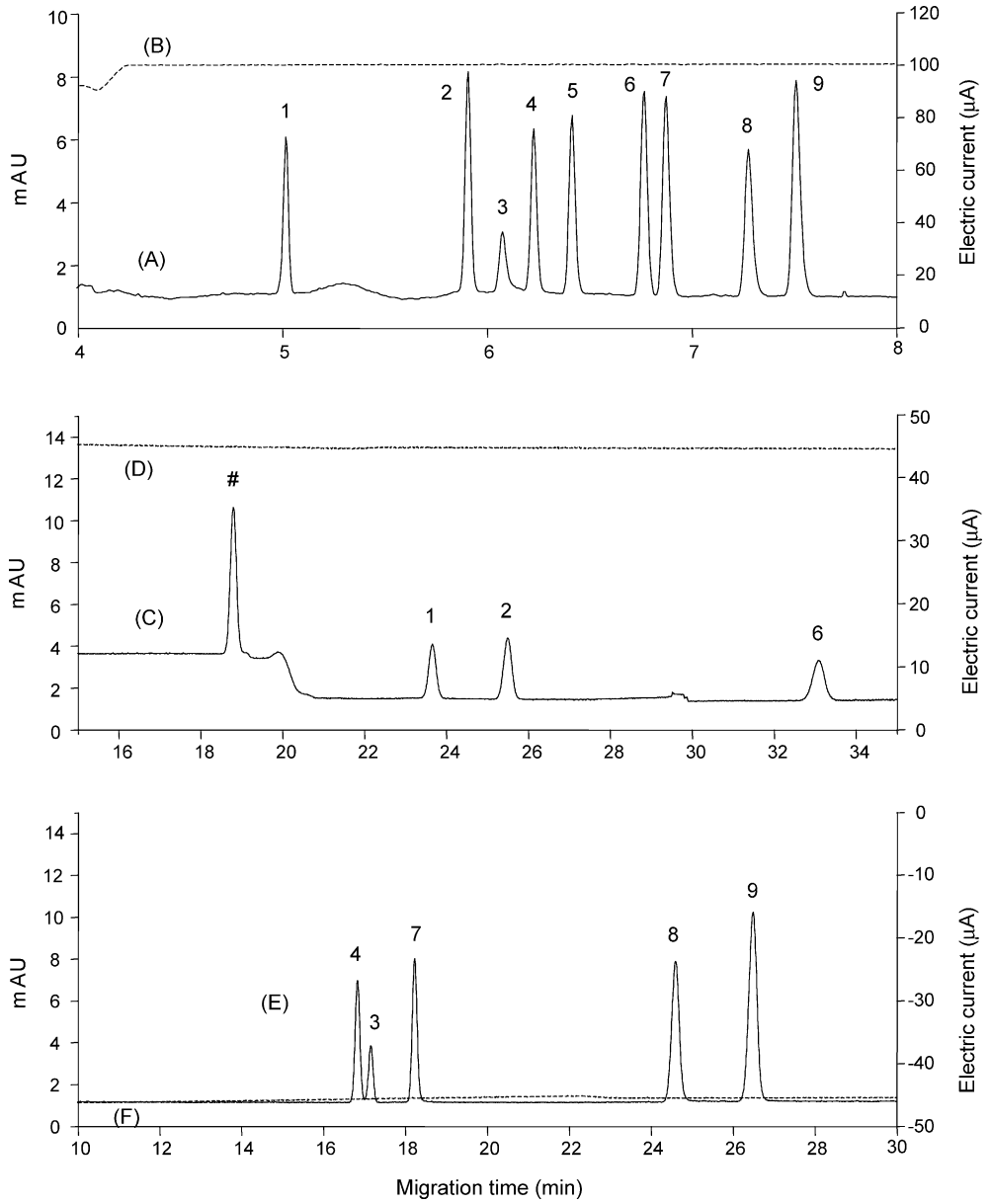

Fig. 1. (A, C, E) Electropherograms of a standard solution in methanol of the nine compounds in study at concentration level of 10 μg/mL. (B) Electric current during the separation process in aqueous media. (D and F) Electric current during the separation process in methanolic media. Capillary: 70 cm × 75 μm I.D.; detection: 210 nm; capillary and sample tray temperature: 25.0 °C; hydrodynamic injection: 50 mbar by 2 s; running buffer: (A) 20 mM aqueous sodium tetraborate at pH 9.6 and (C and E) 20 mM methanolic sodium tetraborate at pH_{app} 9.4; applied voltage: (A–D) +30 kV and (E and F) −30 kV. Peak assignation: (1) 4-Br-3-MeP, (2) 2-Br-4-MeP, (3) PeBP, (4) 2,4,6-TriBP, (5) 2,4-DiBP, (6) 2-BrP, (7) 2,6-DiBP, (8) TBBPA, (9) TCBPA, and (#) EOF marker.

Non-aqueous capillary electrophoresis methods may be developed, using as starting values the buffer composition and pH conditions optimised for aqueous CE separations. Obviously, it has to be considered that electrolytes including organic solvents may have very different chemical and physical properties as compared to aqueous electrolytes, so direct comparison of separations for the same analytes in aqueous running buffers and in organic electrolyte solutions may be difficult or even unfeasible. The pK_a values in water (see Table 1) only gave us an idea for choosing the starting experimental conditions, because the solute pK_a may change for organic solvents by some orders of magnitude, and their values are sometimes unknown. In methanol, the pK_a values of some phenols, e.g. 2,4,6-TriBP are shifted to higher values up to more than four units [37,44]. Optimum electrolyte pH_{app} was determined by testing values in the range between 9.0 and 9.7. Satisfactory results were obtained at pH_{app} 9.4 (see Fig. 1C and E). Working at lower pH_{app} values, compounds like TBBPA and TCBPA were not detected, either in positive or negative polarity mode inside a practical analysis time range, probably because their μ_{eff} were too low or have similar magnitudes to μ_{eo} under these conditions. At higher

pH_{app} values, the analysis time in positive polarity was too long (more than 40 min). On the other hand, in inverse polarity the separation was incomplete, and some compounds coeluted (2,4,6-TriBP and 2,6-DiBP) or were not baseline resolved with PeBP.

In NACE, as compared to aqueous CE, the trend of effective mobility of the analytes has changed. Variations in migration order when methanol was used as running buffer solvent can be attributed to the fact that pH^* is 2.3 units higher than pH_{app} [46], and then the ionization degree was lower for all analytes in comparison to that obtained in aqueous media. Also ion association (with solvent separated ion pairs) and contact ion-pair formation is a phenomenon often occurring in organic solvents with low or moderate dielectric constants [44], and this could contribute to the reduction of μ_{eff}.

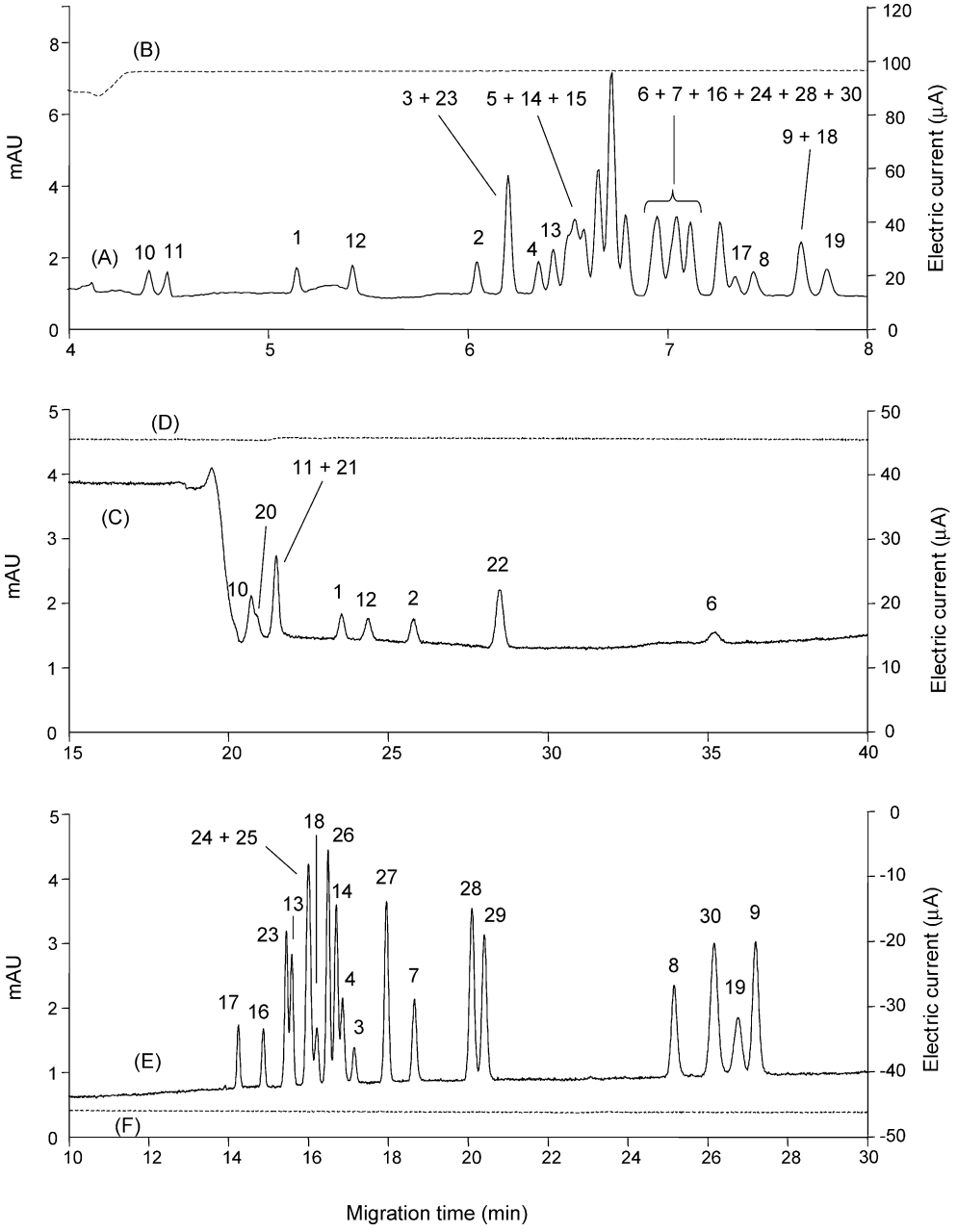

Fig. 2. (A, C, E) Electropherograms of a standard solution in methanol of the mixture of the nine compounds in study and 25 phenol derivatives (enumerated in Section 2.1) in concentration of 2 μg/mL. (B) Electric current during the separation process in aqueous media. (D and F) Electric current during the separation process in methanolic media. CE conditions as in Fig. 1. Peak assignation: (1) 4-Br-3-MeP, (2) 2-Br-4-MeP, (3) PeBP, (4) 2,4,6-TriBP, (5) 2,4-DiBP, (6) 2-BrP, (7) 2,6-DiBP, (8) TBBPA, (9) TCBPA, (10) 2,4-DiMeP, (11) Phenol, (12) 4-CP, (13) PeCP, (14) 2,4,6-TriCP, (15) 2,4-DiCP, (16) 2-Me-4,6-DiNP, (17) 2,4-DiNP, (18) 4-NP, (19) 2-NP, (20) 4-NonylP, (21) BPA, (22) 3-CP, (23) 2,3,5,6-TetraCP, (24) 2,3,6-TriCP, (25) 2,3,4,6-TetraCP, (26) 2,3,5-TriCP, (27) 2,4,5-TriCP, (28) 2,6-DiCP, (29) 2,3,4-TriCP, (30) 2,5-DiCP.

So, under the non-aqueous conditions optimised and inside the investigated pH_{app} range the compounds can be divided into three groups based on their electrophoretic behaviour. The first group consisting of 4-Br-3-MeP, 2-Br-4-MeP and 2-BrP exhibits lower μ_{eff} (absolute values) than μ_{eo}, and therefore, amenable to separations in positive polarity mode (see Fig. 1C); these compounds show the highest increase of mobility with pH_{app}. The second group, consisting of TCBPA, TBBPA and 2,6-DiBP, has mobilities less influenced by pH_{app}. And finally, a third group formed by PeBP and 2,4,6-TriBP with practically constant mobilities. These second and third groups of compounds show higher μ_{eff} (in absolute values) than μ_{eo} (see Table 1) and can be detected applying reverse polarity (see Fig. 1E). 2,4-DiBP

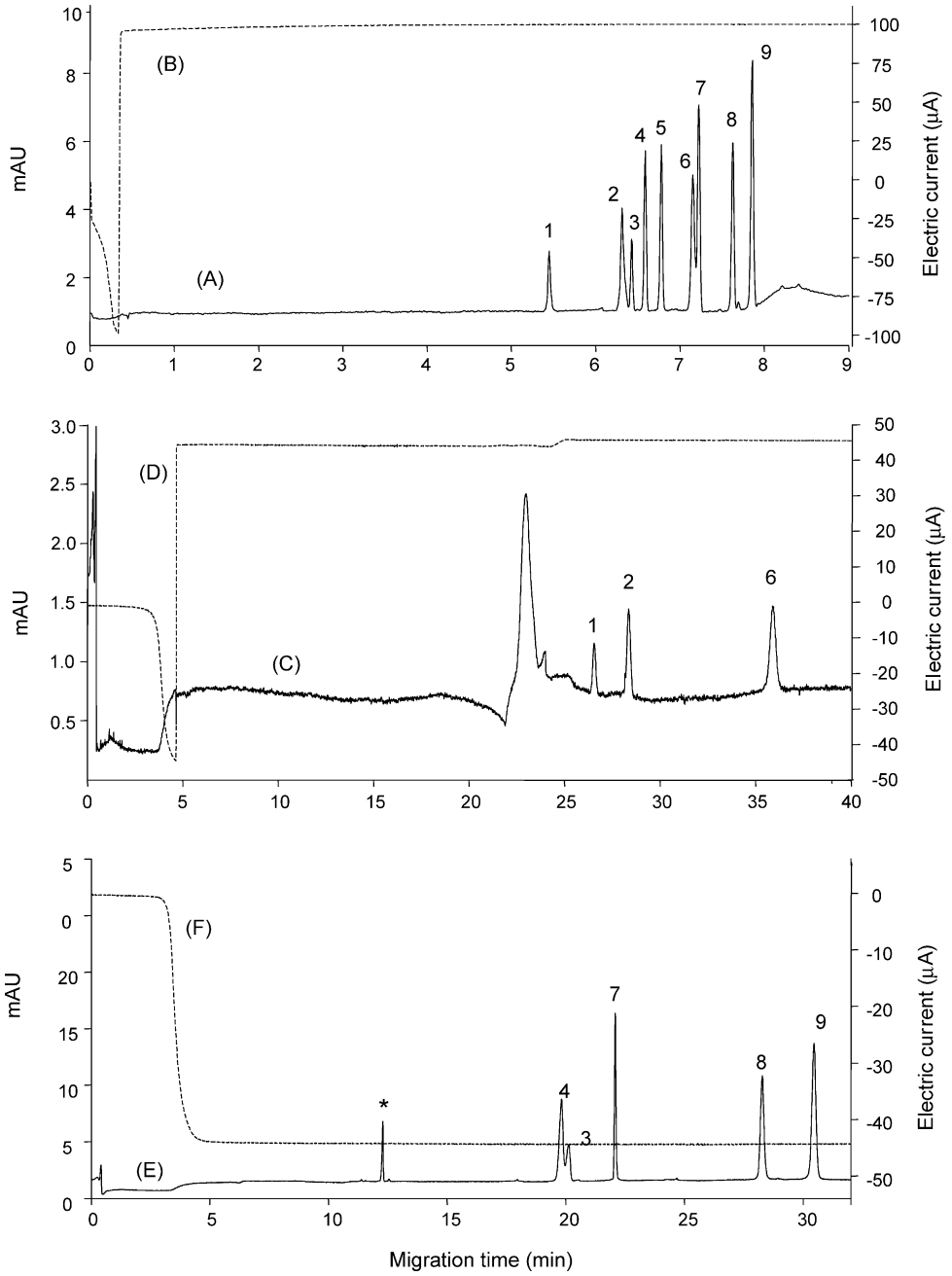

Fig. 3. (A) Electropherogram of a standard solution in methanol of the nine compounds in study in concentration of 0.5 μg/mL. (B) Electric current during the LVSEP process in aqueous media. (C and E) Electropherogram of a standard solution in methanol of the nine compounds in study in concentration of 0.1 μg/mL. (D and F) Electric current during the LVSEP process in methanolic media. Running buffer: (A and B) 20 mM sodium tetraborate at pH 9.6 and (C–F) 20 mM sodium tetraborate at pH_{app} 9.4; applied voltage: (A and B) −30 kV by 0.4 min for removing the matrix and +30 kV in the separation step, (C and D) −30 kV by 4.5 min for removing the matrix and +30 kV in the separation step and (E and F) −30 kV; hydrodynamic injection: (A and B) 50 mbar by 30 s and (C–F) 50 mbar by 300 s. Other CE conditions and peak assignation as in Fig. 1. (*) Tetraborate ions.

was detected only in negative polarity mode at buffer pH_{app} 9.7, due to its low ionization degree below this pH_{app} value.

Three concentration levels of the running buffer were assayed: 20, 30 and 40 mM sodium tetraborate in methanol. As could be expected, the resolution in negative polarity mode was improved with the decrease in the ionic strength because of EOF increasing and the apparent solute mobility decreasing, at the expense of higher analysis time.

3.1.3. Study of potential interferences

To assess the applicability of the developed CE methods in the analysis of bromophenols and halogenated bisphenols in environmental samples, it must be taken into account that many other phenolic compounds (with more or less similar chemical and electrophoretic behaviour) could be present in all kinds of matrix samples. Thus to evaluate the real possibilities of applying the developed methods to real samples a study of several potential interferences was carried out. With this aim, aqueous and non-aqueous CE under the optimal conditions established were applied to the separation of a standard mixture of the bromophenols and bisphenols here studied and a wide variety of phenol derivatives (including phenol, bisphenol A, 17 polychlorinated phenols, 3 nitrophenols, a methylphenol, a nonylphenol and a methylnitrophenol, enumerated in Section 2.1) most of them included in the US Environmental Protection Agency list of priority pollutants and European Union directive 76/464/EEC concerning dangerous substances discharged into the aquatic environment [47,48].

Fig. 2A shows the electropherogram obtained for the analysis in aqueous CE of a mixture containing our analytes and the group of 25 phenolic compounds investigated as potential interferences. As it can be seen in this figure, some peaks on the electropherogram appeared overlapped, and the resolution achieved was far from satisfactory. 4-Br-3-MeP, 2-Br-4-MeP, 2,4,6-TriBP and TBBPA were nicely resolved. 4-NP and TCBPA were not fully separated, although selective detection for nitrophenols could be achieved at 370 nm. However, the other bromophenols comigrated with some chlorophenols, and therefore, could not be determined under these electrophoretic conditions. These results could be expected, due to the sample complexity (34 components) and the structural similarity between some of the phenolic derivatives (several compounds being positional isomers with similar charge to mass ratio).

Comparatively electropherograms obtained in NACE for the analysis of the same complex mixture of analytes and phenolic interferents are shown in Fig. 2C and E. In that case, a good resolution was achieved by using 20 mM sodium tetraborate in methanol at pH_{app} 9.4 as electrophoretic medium. As shown in these figures, the system successfully resolved the compounds of interest, and only 2,4,6-TriBP appeared partially overlapped. Compounds like 2,3-DiCP, 3,5-DiCP and 3,4-DiCP exhibit migration times larger than 40 min using negative polarity. It is evident that the use of non-aqueous BGE provides a significant enhancement in resolution power, thus enabling the application of common widespread UV–DAD for the analysis of such a complex samples.

3.2. Large-volume sample stacking using the electroosmotic flow pump (LVSEP)

In aqueous capillary electrophoresis, the sample was injected during 30 seconds (c.a. 10% of total capillary volume). Under the electric field of reversed polarity (−30 kV), the analytes migrated towards a high conductive buffer zone, while the methanol matrix of low conductivity was simultaneously removed from the capillary by the EOF pump in the opposite direction. Once the capillary was refilled with the running buffer (95% of the buffer conductivity), the anions had been focused into a small zone at the injection end of the capillary. Then, the electrophoretic separation was started by switching polarity to positive mode (approximately 0.4 min after beginning the analysis time). It has been shown that increasing injection time over 60 s causes significant losses in resolution.

Table 2
Limits of quantification (LOQs) and enhancement in sensitivity (concentration factor) using sample stacking in both aqueous and non-aqueous CE

Compound	Limits of quantification[a] (μg/L)					
	Aqueous CE			Non-aqueous CE		
	CE-DAD[b]	LVSEP–CE-DAD[c]	Concentration factor	NACE–DAD[d]	LVSEP–NACE–DAD[e]	Concentration factor
4-Br-3-MeP	1095	234	5	1754	110.6	16
2-Br-4-MeP	364	70	5	1396	76.5	18
PeBP	935	124	8	450	5.5	82
2,4,6-TriBP	566	49	12	416	4.6	90
2,4-DiBP	487	47	10			
2-BrP	835	47	18	2287	70.7	32
2,6-DiBP	801	31	26	433	1.3	333
TBBPA	683	42	16	485	1.6	303
TCBPA	468	28	16	280	1.3	215

[a] LOQ: ratio signal-to-noise (S/N) of 10; detection at 210 nm, except for PeBP at 230 nm.
[b] CE conditions as in Fig. 1A.
[c] CE conditions as in Fig. 3A.
[d] CE conditions as in Fig. 1C and E.
[e] CE conditions as in Fig. 3C and E.

When analyses were performed in non-aqueous CE system, the capillary was (c.a. 95%) filled with the sample employing 300 s of hydrodynamic injection time. Since methanol was used as the running buffer solvent, appropriate suppression of EOF made LVSEP possible without polarity switching (except for those analytes with lower μ_{eff} than μ_{eo} that must be analysed in positive polarity, see Table 1; in this case, the LVSEP process was carried out with polarity switching like in aqueous media). Thus, after injection, before removing of the methanol matrix and stacking of the anions, the capillary was refilled with the running buffer of high conductivity, and the overall EOF became further reduced due to the increased ionic strength. When the EOF and the electrophoretic velocities of analytes were balanced, the migration direction of the stacked analytes was self-switched toward the detector by their own electrophoretic mobility, while the reduced EOF moved in the opposite direction, and thus the separation of the highly stacked sample occurs.

Fig. 3A, C and E show the separation and the enhancement in sensitivity achieved using LVSEP, in comparison with normal hydrodynamic injection (see Fig. 1A, C and E, respectively). The process of removing the methanol plug out of the capillary imposed the differences in migration times between the electropherograms in Figs. 1A and 3A, and between the electropherograms in Figs. 1C and E, 3C and E. Borate ions in the inlet buffer are also injected hydrodynamically and stacked at the concentration boundary while the methanol plug is being removed [49].

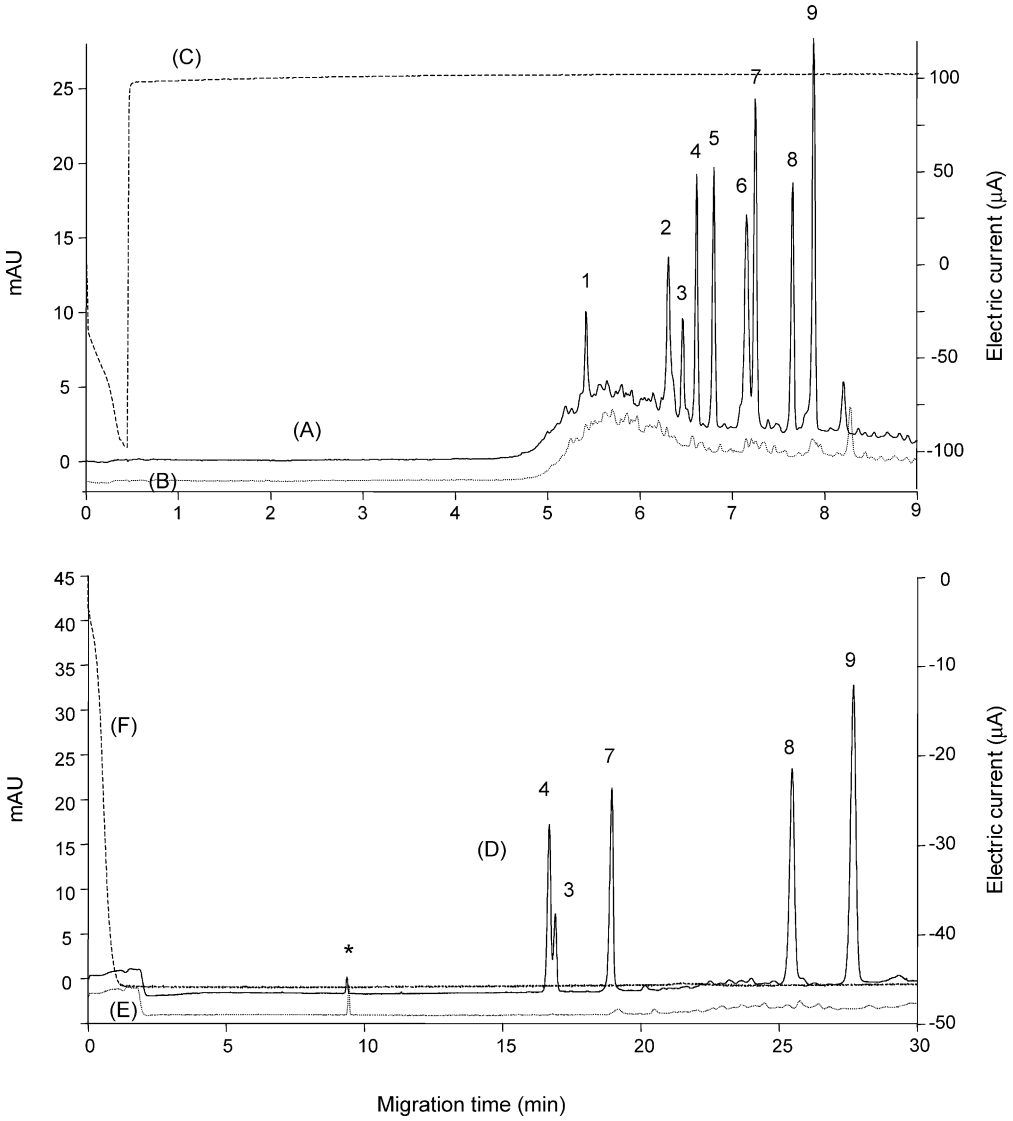

Fig. 4. Electropherograms obtained from SPE extracts of (A and D) 500 mL of wastewater sample spiked at a concentration of 12 μg/L for all compounds, and (B and E) 500 mL of raw wastewater sample. (C) Electric current during the LVSEP process in aqueous media. (F) Electric current during the LVSEP process in methanolic media. Hydrodynamic injection: 50 mbar by 30 s; running buffer: (A–C) 20 mM sodium tetraborate at pH 9.6 and (D–F) 20 mM sodium tetraborate at pH_{app} 9.4; applied voltage: (A–C) −30 kV by 0.4 min for removing the matrix and +30 kV in the separation step, and (D–F) −30 kV. Other CE conditions and peak assignment as in Fig. 1. (*) Tetraborate ions.

Fig. 3B, D and F shows the electric current during the LVSEP process. The intensity of the electric current increased rapidly (absolute values), while the sample matrix was removed and the ionic strength of the medium in the capillary increased close to the value registered when the capillary was filled with the running buffer. Note that the polarity was switched in that moment for the separation in aqueous CE (see Fig. 3B) and in NACE separations in positive polarity (Fig. 3D).

The sensitivity improvement achieved by LVSEP was evaluated through the increase in detector response by comparing normal hydrodynamic injections (50 mbar by 2 s) and sample stacking injections (50 mbar by 30 s or 300 s, in aqueous or non-aqueous CE, respectively). Table 2 shows the estimated values for the limits of quantification (LOQs) in both techniques. Coupling LVSEP to CE improved the LOQs about one and two orders of magnitude in aqueous CE and NACE, respectively. This allows the application of the procedure for samples in the μg/L level using conventional UV absorption detection.

3.3. Analysis of water samples

For the application of developed procedures to real wastewater samples, the analytes were extracted using SPE Oasis cartridges, as it has been described in Section 2.5. Employing methanol as solid-phase eluent, the SPE extracts of water samples can be directly injected into the CE system, making the extraction process compatible with the electrophoretic analysis.

As it has been reported previously [35], in NACE matrix components affects the ionic strength of sample extracts so the removing time of sample plug in the stacking process in methanolic medium increased. As a consequence, injection time had to be reduced to a level enabling quantitative injection of analytes in wastewater samples [35]. Using injection times of 30 s, comparable results were attained in both LVSEP–CE and LVSEP–NACE. As an example, Fig. 4 shows the electropherograms obtained in the analysis of a wastewater SPE extract using both aqueous and non-aqueous CE methods under the described conditions.

4. Conclusions

CZE methods based either on aqueous and non-aqueous running buffers, for the separation and quantification of phenolic and bisphenolic compounds in environmental samples were developed and optimized. Both aqueous electrophoresis (CE) and non-aqueous electrophoresis (NACE) methods were suitable for the analysis of compounds under study. The separation of the analytes from many potential interferents was demonstrated with NACE method.

Using LVSEP, for the in-line concentration of the analytes, quantification limits in the range of μg/L levels may be achieved with conventional UV absorption detection. Additionally, the application of SPE sample preconcentration allows the analysis of the studied bromophenols and halogenated bisphenols in wastewater and other water samples of environmental concern.

Employing methanol as eluent in SPE, the extracts containing the analytes can be directly injected into the capillary electrophoresis system. It allows a good compatibility between extraction and electrophoretic processes, simplifying the whole analytical process.

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