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On-line flow sample stacking in a flow injection analysis–capillary electrophoresis system: 2000-fold enhancement of detection sensitivity for priority phenol pollutants

Petr Kuban, Maria Berg, Carlos García[1], Bo Karlberg*

Department of Analytical Chemistry, Stockholm University, Svante Arrhenius Vag 10–12, SE-10691 Stockholm, Sweden

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Abstract

A flow injection analysis–capillary electrophoresis system has been used for on-line flow stacking of 11 US Environmental Protection Agency priority phenol pollutants. Samples containing low concentrations of phenols dissolved in deionised water are continuously delivered to the capillary opening by means of a peristaltic pump. The sample components stack at the boundary between the highly conductive separation electrolyte and the introduced sample. By selecting an appropriate electrolyte and stacking conditions the movement of the electrolyte solution inside the capillary can be reduced, thereby improving the stacking efficiency. The electrolyte used here contained 20 m*M* phosphate, 8% 2-butanol, and 0.001% hexamethonium bromide at pH 11.95, and the stacking was carried out at 2 kV for 240 s. These conditions allowed up to 2000-fold preconcentration of the selected phenols. No matrix removal was necessary. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Sample stacking; Sample handling; Flow injection analysis; Phenols; Chlorophenols

1. Introduction

The poor sensitivity of capillary electrophoresis (CE) to low sample concentrations is due to the extremely small amounts of the sample injected. It is one of the most frequently cited drawbacks for this separation technique, which is progressive in many other respects. In fact, manual or semiautomatic procedures are often used to pre-treat the sample, and often to enrich the analytes of interest in a small volume, part of which is subsequently injected into the CE system. Recently, several research groups have introduced systems in which an on-line sample treatment step precedes the CE separation. Such systems can reduce the error in manual handling and can also be used in an automated fashion [1–4]. These systems usually involve the hyphenation of one or more analytical techniques to CE and can be utilised for a large variety of preconcentration applications.

As early as 1979, Mikkers et al. [5] noticed that the sensitivity of CE analysis was enhanced for electrokinetically injected samples prepared in a

*Corresponding author. Tel.: +46-8-162-437; fax: +46-8-156-391.

E-mail address: bo.karlberg@anchem.su.se (B. Karlberg).

[1]Present address: INFIQC Departamento de Fisioquímica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina.

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matrix with low conductivity relative to the separation electrolyte (SE). In a later series of papers, Chien and co-workers [6–10] theoretically explained and practically demonstrated the phenomenon of field amplified sample stacking (FASS). Using their concept, a part or even the entire separation capillary is initially filled with sample dissolved in a low conductivity buffer (LCB) or deionised water. The ions from the sample stack at the LCB–SE boundary due to the drop in the electric field intensity across it, in accordance with Ohm's law. The separation is then started. However, a large volume of the sample matrix, present in the separation capillary after sample stacking, evokes a mismatch in the local electroosmotic flow (EOF) velocities and induces laminar flow inside the capillary. Thus, after completion of the stacking procedure, the low conductivity sample matrix must be removed. This can be accomplished by switching polarity [10] or by applying back-pressure [11]. Matrix removal, however, requires exact control of the process, since incorrect handling can cause analyte losses and reduce precision. Another approach is to utilise the EOF as a pump [12]. Recently, He and Lee [13] suggested a mechanism similar to the EOF pumping principle using a separation electrolyte with either a low or a high pH for pre-concentration of some small organic and inorganic anions. Applying any one of the above-mentioned methods, FASS becomes an efficient concentration technique and analytes can be pre-concentrated ten to 100-fold.

Chien and Burgi [8,9] suggested an alternative to FASS, called head column field amplified sample stacking (HC-FASS). This technique relies on the introduction of a short sample plug prepared in LCB or water into the separation column and stacking from the entire sample volume in the vial. The authors recommend injection of a short water plug before the stacking to improve the performance of HC-FASS. Obviously, this technique has several advantages over the FASS method. First, since the introduced sample volume is relatively low in comparison to the total capillary volume, the laminar flow, caused by the mismatch of the EOF velocities, is small, and removal of the sample matrix is not necessary if relatively short stacking times and voltages are applied. Furthermore, the stacking occurs from the whole sample volume in the vial, so it is not limited by the capillary volume. Thus, higher preconcentration factors can be achieved. Zhang and Thormann [14,15] have demonstrated up to a 1000-fold concentration of positively charged hydrophobic compounds in their model system after fine-tuning the sample/electrolyte composition (by adding organic solvent and small amounts of H^+) and optimising the sampling time/voltage. The cited authors also found that it was necessary to introduce a short water plug before the stacking started, and to reduce the product of the high voltage and injection time, to prevent excessive band broadening.

Kuldvee and Kaljurand [16] were the first to demonstrate sample stacking from a flow by using a laboratory-made pneumatic sampling device coupled to the CE system. By pressurising the sample vial, a fraction of the sample is forced to enter the capillary through a combined electrokinetic/hydrodynamic injection mechanism. The sample enters the capillary and at the same time the analytes are concentrated at the sample–electrolyte boundary region. A 100-fold concentration factor was achieved by the cited authors for BTEA and BTBA cations in a 10-s stacking mode at 18 kV compared to traditional electrokinetic injection. The time limitation of the stacking step was mainly due to the excess of sample introduced by hydrodynamic forces during the stacking process. Also, due to the contribution of pressure-driven hydrodynamic injection, the flow profile at the sample–electrolyte boundary is probably of a laminar nature, which could have an adverse effect on the efficiency of the pre-concentration. The excess of the sample matrix entering the capillary can be efficiently removed by applying counter-pressure during or after the stacking step [17]. Nevertheless, the laminar-type profile of the boundary remains a problem and limits the preconcentration efficiency by band broadening.

Recently, Quirino and Terabe [18] presented a two-step approach to concentrate positively chargeable analytes. The sample is first accumulated inside the capillary by field enhanced sample injection (FESI). The broad zone of the analytes is then swept into a narrow zone by a micellar pseudo-stationary phase (sweeping) and further separated in the micellar electrokinetic chromatography (MEKC) mode. The authors approached a million-fold improvement in peak heights compared with usual injection. In

this paper we present an on-line sample stacking technique in a flow-injection analysis (FIA)–CE system, which achieved up to 2000-fold pre-concentration of the priority phenol pollutants.

2. Experimental

2.1. Instrumentation

A modification of the FIA–CE system previously presented by Kuban et al. [1] was used for sample stacking from a flow (Fig. 1).

2.1.1. The FIA system

Two eight-channel peristaltic pumps (Gilson, France) were used to continuously deliver electrolyte and sample solutions, respectively. All parts of the FIA manifold system were connected via PTFE tubing (0.7 mm I.D.).

2.1.2. The FIA–CE interface

An FIA–CE interface [1] was used to introduce samples into the separation capillary, electrokinetically, from the sample flow.

2.1.3. The CE components

The CE part of the system was accommodated in a homemade plexiglass box equipped with a safety lock on the access door for protection. It consisted of a CZE 3000 high-voltage supply (Spellman, Plainview, NY, USA) and two platinum electrodes positioned in the flow channel and outlet vial. A potential of 25 kV was applied during separations. The stacking was performed at a potential of 2–6 kV. Polyimide coated capillaries were used (50 μm I.D.×375 μm O.D.; Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillary was 70 cm and the length from the injection to the detection points was 50 cm.

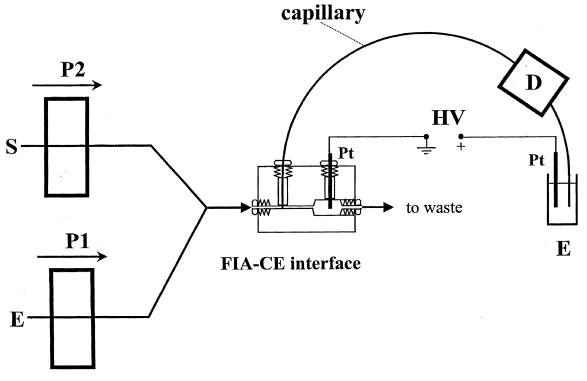

Fig. 1. Diagram of the FIA–CE system for sample stacking from a flow. D, detector; E, electrolyte; HV, high-voltage supply; P1, P2, peristaltic pumps; Pt, platinum electrodes; S, sample.

2.1.4. The detection system

An ISCO CV^4 UV–visible detector (ISCO, Lincoln, NE, USA) was used for direct UV detection at 210 nm. Electropherograms were registered using an ELDSWin Pro laboratory data system (Chromatography Data Systems, Kungshög, Sweden).

2.2. Operation of the system

The system comprises of two peristaltic pumps (P1, P2) which intermittently deliver either the electrolyte or the sample solutions. During the stacking step, pump P1 is stopped, while pump P2 delivers the sample to the FIA–CE interface. A high voltage of 2 kV is applied during the stacking. When the stacking procedure is finished, the high voltage is interrupted, pump P2 is stopped and pump P1 is resumed. A voltage of 20 kV is now applied and the separation takes place. The whole sampling procedure takes ~5 min and ~2 ml of the sample is needed for each run.

2.3. Chemicals

All reagents were of analytical grade and deionised water with a resistivity greater than 18 MΩ cm^{-1} was used. The carrier electrolyte consisted of 20 m*M* phosphate, 8% 2-butanol and 0.001% hexamethonium bromide (HDB) at pH 11.95. The electrolyte was prepared daily by diluting stock solutions of 200 m*M* sodium phosphate, 0.2% HDB and butanol. The pH of the electrolyte was adjusted with 0.1 *M* NaOH. The electrolyte was filtered and degassed prior to use. Stock solutions of phenols, 1000 μg ml^{-1}, were prepared in methanol (spectroscopy grade) and kept at low temperature (+4°C). The working solutions of the phenols were prepared

by diluting the respective stock solutions with running buffer or deionised water.

The capillary was conditioned daily by flushing with electrolyte for 30 min and the closing down procedure consisted of rinsing with deionised water for 10 min and air for 5 min. The capillary was always stored dry overnight.

3. Results and discussion

3.1. Band broadening in sample stacking

To optimise the stacking efficiency several factors have to be taken into consideration. Peak broadening is one of the most serious problems when mixtures of several closely migrating analytes are to be stacked and subsequently separated. The peak variance during the stacking in CE can be expressed as:

$$s^2 = s_d^2 + s_i^2 + s_a^2 + \Sigma s_j^2 \quad (1)$$

where s_d^2 is the diffusional variance, s_i^2 the variance caused by the injection plug length, s_a^2 the variance due to the laminar flow and Σs_j^2 the variance due to other factors.

Substitution according to Burgi and Chien [7] results in:

$$s^2 = 2Dt + \frac{x_\alpha^2 L^2}{12\gamma^2} + \left[\frac{x_\alpha(\gamma.v_{eo1} - v_{eo2})}{\gamma.x_\alpha + (1 - x_\alpha)}\right]^2 \cdot \frac{r_0^2 t}{24D} \quad (2)$$

where D is the normal diffusivity of the sample ions, t is the injection time, x_α is the ratio of the injection plug length to the length of the column, x_i/L, γ is the field enhancement factor (which can be approximated to the ratio between the conductivities of the electrolyte and sample solutions), v_{eo1} and v_{eo2} are the electroosmotic flow velocities in the sample and electrolyte zones, respectively, and r_0 is the inner diameter of the capillary.

From this equation it follows that the band broadening during the stacking step depends on the injection time, t, injection plug length, x_α, and ratio of conductivities, γ, between the LCB and SE. Burgi and Chien [7] showed that sharpening of the stacked zone and the laminar flow induced by the mismatch in the EOF velocities counteract each other, and they found an optimum at $\gamma = 8$ for their model system. Fig. 2 shows graphs for the band broadening effect versus the injection plug length (A) and the field enhancement factor (B). The plots are based on the following values inserted in Eq. (2): $D = 9 \cdot 10^{-5}$ cm^2 s^{-1}, $t = 120$ s, $L = 70$ cm, $v_{eo1} = v_{eo2} = 0.2$ cm min^{-1}, $r_0 = 0.0025$ cm. As can be seen, the peak broadening expressed as s^2 (cm) is much more strongly influenced by the increase in the sample plug length, x_α (Fig. 2A) than by the ratio of the sample/electrolyte conductivities, γ (Fig. 2B). Special care should therefore be taken to minimise the length of the injected sample plug. In electrokinetic injection the sample plug length, x_α, is defined as:

$$x_a = (\mu_i + \mu_{eof})_{inj} E.t.V_{inj} \quad (3)$$

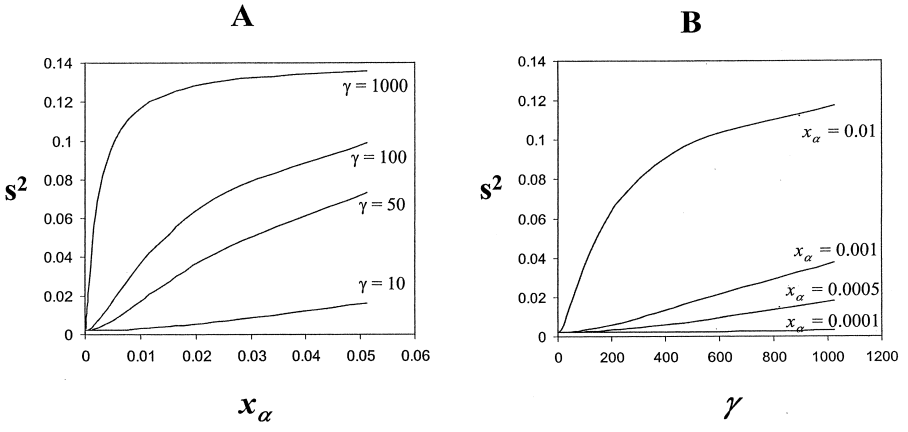

Fig. 2. Plot of the theoretical influence of sample plug length (x_a) and field enhancement factor (γ) on the peak width variance (s^2).

where μ_i is the electrophoretic mobility of an ion, μ_{eof} the electroosmotic mobility, E the electric field strength, t the injection time and V_{inj} the injection voltage.

Therefore, to decrease the band broadening, factors such as high voltage (V_{inj}), injection time (t), and EOF velocity should be optimised. To achieve high stacking efficiency, on the other hand, the product of the applied voltage and time should be maximised.

3.2. System optimisation

The EOF velocity in the system should be selected to balance the need to ensure sufficient separation efficiency and analysis time, on the one hand, and the requirements for optimal sample stacking on the other. Important aspects of EOF modification will be discussed later. In the optimisation experiment, three factors were considered: injection time, high voltage and sample flow-rate. The peak height and area of three phenols (2-NP, 2,4-DNP and 2-CP) were chosen as response functions. A two-level full factorial design was then constructed, including three experiments in the center point ($2^3+3=11$). Each experiment was repeated three times and the mean response values were evaluated by multiple linear regression (MLR). The experimental domain is shown in Table 1.

The highest sensitivity was achieved when long injection times were combined with high-voltage values. However, increased peak broadening was observed at the highest voltage value, 6 kV, but no increase in peak height. This led to the conclusion that lower high-voltage (HV) values are preferable. In conclusion, a HV of 2 kV and an injection time of 120 s were found to give the best results. The injection time could be further increased outside the selected experimental domain when analysing compounds present at concentrations close to their limits of detection. Interestingly, there was no response at all when sample flow-rates were too high. This is not fully understood, but it is possible that the turbulent mixing of the sample and electrolyte at the capillary aperture that is likely to occur at high flow-rates could disturb the flat boundary profile and interfere with the stacking process. Also, high sample flow-rates may give rise to under-pressure in the capillary, thus removing the stacked analytes.

Table 1
Factors and levels used for optimising stacking conditions

Factor	Unit	Low	High
Flow rate	(ml min^{-1})	0.4	1.8
High voltage	(kV)	2	6
T_{inj}	(s)	30	120

3.3. Introduction of a water pre-plug

The FIA–CE approach for introducing samples differs markedly from that normally applied in either commercial or homemade CE instruments. Electrolyte is always present in the FIA–CE flow system, although the propelled sample temporally replaces this electrolyte solution during the stacking procedure. This is in contrast to the method of introducing samples used in commercial instruments, in which the sample vial physically replaces the electrolyte vial when injecting the sample. A further difference relates to the mode of the HV supply for the two types of system; when applying the FIA–CE approach, the HV is uninterrupted, while in the conventional approach, a disruption occurs during the exchange of vials.

Several research groups state the necessity of introducing a short water plug into the capillary prior to the stacking to avoid erroneous and irreproducible results or damage to the capillary. The problems observed in the absence of a water pre-plug have been attributed to the disturbance in the sample–electrolyte boundary caused by the withdrawal/insertion of the capillary tip from sample/electrolyte vials. Occasionally, fusing of the capillary could also occur. In our experiments we have not encountered any serious problems with disturbance at the sample–electrolyte boundary since there is no physical movement of the capillary or hydrodynamic flow inside the capillary and it was not necessary to introduce any water pre-plug before the stacking step. Some authors have observed an increase in pre-concentration efficiency and reproducibility when using a water pre-plug [14,15], while others have not observed any significant effect [18]. In our experiments we have not seen any increase in efficiency when a water pre-plug was introduced into the capillary. On the contrary, when a pre-plug was

introduced by hydrodynamic means, the stacking efficiency decreased, probably again due to the disturbance induced by the hydrodynamic flow at the sample–electrolyte boundary. In the optimisation of the stacking procedure, we observed capillary fouling when too high voltages (10–20 kV) were used during the stacking step. Removal of a small part of the capillary inlet eliminated this problem, which indicates that some capillary fusing might have occurred. When using lower voltages for the stacking (2–6 kV) no capillary fouling was observed.

3.4. Separation of 11 US Environmental Protection Agency (EPA) priority phenols

A total of eleven EPA-listed substituted phenols were selected to provide a model mixture to demonstrate the application of stacking from the flow. The maximum allowed levels of these phenols in publicly supplied water are 0.5 μg ml^{-1} [19].

Capillary electrophoretic methods for separating phenols offer sufficient selectivity but lack the required sensitivity. Therefore, various sample preconcentration methods including solid-phase extraction (SPE) on C_{18} or graphitised carbon, and sample stacking have been applied. Stacking methods have been used with phenols in the counter-electroosmotic separation mode, for instance [19,20]. Recently, Zemann and Volgger [21] suggested a co-electroosmotic separation of various phenols. In their system, the capillary wall was coated with HDB to give it a positive charge and reverse the EOF. Various amounts of different organic modifiers (up to three) were added to the electrolyte to alter the separation selectivity, and 11 EPA phenols could be separated in less than 3 min. No systematic study of the influence of the organic modifiers was performed, and they were added according to ''trial and error''. In our investigations we adopted the co-electroosmotic separation mode suggested by Zemann and Volgger [21]. First, by employing a low concentration of HDB, the EOF was reversed. The optimum HDB concentration was 0.001%. Lower concentrations resulted in irreproducible migration times during the first few runs, until an equilibrium was reached. The decrease in migration times in consecutive runs indicated that at low concentrations of the EOF modifier the surface of the fused-silica capillary is not saturated, and in each consecutive run more molecules of HDB bind to the capillary wall and increase the reversed EOF. The steady state EOF value was the same as the value reached with an HDB concentration of 0.001%. This concentration was therefore used in all subsequent experiments.

Since bubble formation in the flow parts of the FIA–CE system is pronounced when electrolytes containing significant amounts of organic solvents are used, the next step in system refinement was to separate the selected 11 EPA phenols in the simplest possible electrolyte system. Binary water–alcohol mixtures seemed to be a suitable choice. Several solutes including methanol, propanol, 1-butanol and 2-butanol were tested, but the best results were obtained with 2-butanol. The effect of varying the concentration of 2-butanol in the electrolyte is shown in Fig. 3. As can be seen, co-migration of several phenols, especially 2-CP, 2-NP and phenol, occurs at both the low and high ends of the curves, while satisfactory separation is achieved with a 2-butanol concentration of 8%. In Fig. 4 the effect of pH on the separation of the selected phenols can also be observed. A pH value of 11.95 provides the maximum separation selectivity. An electropherogram of the separation of the 11 phenols in an electrolyte containing 8% 2-butanol at this pH is depicted in Fig. 5A.

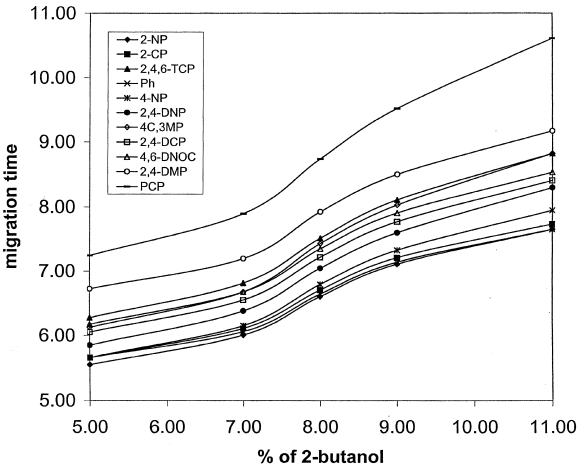

Fig. 3. Influence of the concentration of 2-butanol on separation of the 11 phenols. Electrolyte: 20 m*M* phosphate, 0.001% HDB, pH 12. Separation voltage: 25 kV, detection: 210 nm.

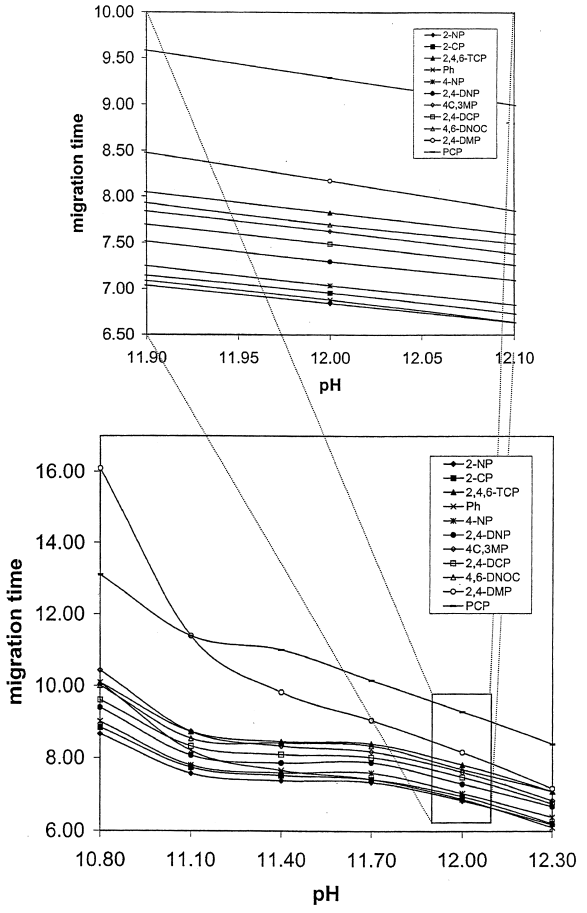

Fig. 4. Influence of pH on separation of the 11 phenols. Electrolyte: 20 m*M* phosphate, 8% 2-butanol, 0.001% HDB. Separation voltage: 25 kV, detection: 210 nm.

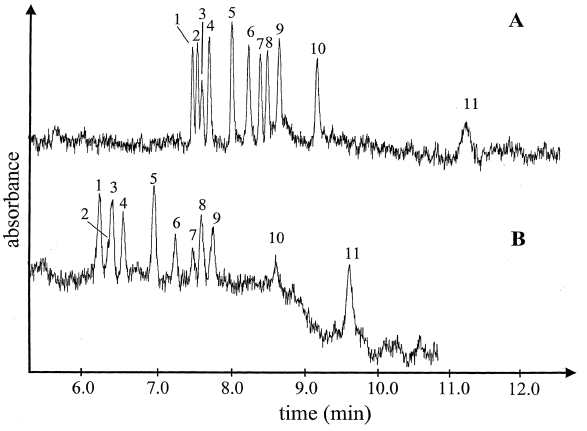

Fig. 5. Comparison of sensitivity of determination of the 11 phenols by hydrodynamic injection (A) and sample stacking (B). Conditions: (A) HD injection for 60 s at 15 cm, sample: 11 phenols, 3–20 ppm. (B) Sample stacking for 240 s at 2 kV, sample: 11 phenols, 0.6–4 ppb. Electrolyte: 20 m*M* phosphate, 8% 2-butanol, 0.001% HDB, pH 11.95. Separation voltage: 25 kV, detection: 210 nm. The migration order is the same as in Table 2.

3.5. Determination of limits of detection

The optimised conditions identified for sample stacking from the previous experiments were adopted for determining the limits of detection (LODs) for the 11 EPA phenols. First, the LOD values were determined using hydrodynamic injection. The phenols, prepared in the separation electrolyte, were injected by gravity into the separation capillary for 60 s at a height of 15 cm. Further increases in the injection time or height caused excessive broadening of the peaks of the phenols rather than an increase in the peak heights. For these injection conditions, LOD values in the range 0.7–4.3 μg ml^{-1} were found (Table 2). This is insufficiently sensitive for determining the concentrations allowed in publicly supplied water. A 5000-fold diluted solution of the phenols was then subjected to the pre-concentration step, consisting of stacking at 2 kV for 240 s. In this manner, the LOD values could be decreased by a factor varying between 250 and 2000 for individual phenols, as shown in Table 2. The separation at the low ppb levels is demonstrated in Fig. 5B. As can be seen, the sensitivity of the peaks is amplified more than 1000-fold for most of the phenols. It should be mentioned that due to the long pre-concentration time, some peak broadening can be observed which decreases the separation of closely migrating phenols, but nevertheless ten of the 11 phenols can be separated. The peak broadening is probably due to diffusional migration of the analytes from the stacked zone into the electrolyte during the stacking step. Zhang and Thormann [15] suggested that a high conductivity pre-plug could be injected prior to the stacked sample in HC-FASS to further improve the enrichment, by trapping the analytes in a sharp zone before the separation starts. We have tried adopting their approach, but the separation with a high conductivity pre-plug failed. In this context, the double-stacking procedure (FESI–sweep), presented

Table 2
Limits of detection, pre-concentration factors (F) and repeatability data

Analyte	LOD (HD[a]) (μg ml^{-1})	LOD (ST[b]) (ng ml^{-1})	F	RSD (%)
2-Nitrophenol (2-NP)	2.12	1.06	2000	8.4
2-Chlorophenol (2-CP)	0.70	0.70	1000	7.2
2,4,6-Trichlorophenol (2,4,6-TCP)	1.21	1.21	1000	9.6
Phenol	1.86	3.72	500	10.2
4-Nitrophenol (4-NP)	3.72	3.72	1000	8.2
2,4-Dinitrophenol (2,4-DNP)	4.00	4.00	1000	9.4
4-Chloro-3-methylphenol (4-C-3-MP)	1.16	2.32	500	13.3
2,4-Dichlorophenol (2,4-DCP)	1.06	1.06	1000	7.4
4,6-Dinitro-*ortho*-cresol (4,6-DNOC)	3.80	3.80	1000	10.8
2,4-Dimethylphenol (2,4-DMP)	1.13	4.52	250	6.7
Pentachlorophenol (PCP)	4.24	2.12	2000	8.3

[a] Hydrodynamic injection for 60 s at 15-cm height.
[b] Sample stacking from the flow at 2 kV for 240 s.

by Quirino and Terabe [18], for efficient preconcentration of analytes in MEKC, could be a useful alternative to solve the problem of sample diffusion into the buffer zone. Table 2 also shows the repeatability data ($n=7$, $<14\%$ RSD) obtained for the stacking of the standard solution of 11 phenols, which were satisfactory for the concentration ranges used.

4. Conclusions

The FIA–CE system has shown a promising capacity for automated sample pre-concentration using stacking from the flow. The flow of diluted sample provides a constant supply of fresh sample to the capillary aperture. This circumvents the problems associated with sample overheating, including overheating inside the capillary, when moderate voltages are applied. Since the injection and sample plug advancement in the capillary is driven purely by the electroosmotic flow, no disturbance of the sample–electrolyte boundary due to the pressure-induced flow is observed. In optimised stacking conditions, a 2000-fold pre-concentration of phenolic pollutants can be achieved. The technique might also have potential for combined sample concentration methods such as SPE–stacking and combined FESI–sweep.

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