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Analysis of glyphosate using capillary electrophoresis with indirect detection

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Abstract

A method has been developed for the analysis of glyphosate and aminomethylphosphonic acid (AMPA) in water. Using a pH 7.5, 10 m*M* phthalate background electrolyte containing 0.5 m*M* tetradecyltrimethylammonium bromide (TTAB) as an electro-osmotic flow modifier, the analytes were separated in less than 4 min under reverse polarity conditions and detected indirectly. Response was shown to be linear for over two orders of magnitude, calibration in the range 0.01–1.0 m*M* (1.7–170 μg ml^{-1} glyphosate, 1.1–110 μg ml^{-1} AMPA) giving least squares correlation coefficients of 0.9998 and 0.9999. Precisions of migration times and normalised peak areas were typically less than 0.7 and 2.0% respectively. Calibration slopes gave transfer ratios for glyphosate (0.98) and AMPA (0.75) in good agreement with values computed from theory using coupled transport equations. With stacking from water, the limit of detection for glyphosate was 5 μM, i.e. 0.8 μg ml^{-1} (twice peak-to-peak noise) for a 6.7 nl injection, calculated from a 0.05 m*M* standard solution. Field-amplified sample injection has enabled 0.01 μM (2 ng ml^{-1}) to be detected, a factor of ~1000 in signal enhancement being obtained over conventional hydrodynamic injection with no stacking.

Keywords: Environmental analysis; Glyphosate; Aminomethylphosphonic acid; Pesticides

1. Introduction

Glyphosate [N-(phosphonomethyl)glycine] [1–3] is a non-selective herbicide for control of long grasses and broad-leafed weeds. It has low soil residual activity, being rapidly and nearly completely adsorbed by soil, and consequently is only effective when used post-emergence. Once applied to vegetation, it is absorbed by the leaves and translocated throughout the plant tissue. Here it inhibits the action of the enzyme 5-enolpyruvate-shikimate-3-phosphate-synthase (EPSP synthase) in the shikimate pathway, which produces aromatic amino acids such as phenylalanine for protein synthesis and other secondary plant products. Although glyphosate mainly targets EPSP synthase, photosynthesis and respiration are also affected. Reaction, however, is relatively slow, with treated plants taking at least seven days to exhibit any effects and up to three weeks to die.

Glyphosate is widely used all over the world for a number of pre-plant, post-harvest and non-crop applications. Despite its low mammalian toxicity, since EPSP synthase is not found in animals, the fact that glyphosate has been found to cause reproductive disorders [4,5] is of particular concern as treatments applied close to harvest time could lead to substantial residues in the harvested crop. The World Health Organisation has evaluated some 230 pesticides for which acceptable daily intakes have been allocated [5]; currently for glyphosate this is 0.3 mg kg^{-1}

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body mass. Thus, there is a need to determine glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) in a variety of samples matrices. The effectiveness of glyphosate as a herbicide has been partially due to its chemical nature; high water solubility, insolubility in organic solvents, complexing behaviour and similarity to naturally occurring amino acids and amino sugars. However, it is these properties that also make the analysis of glyphosate, and indeed AMPA, difficult, especially when present at residue levels in a variety of matrices. Lengthy extraction and clean-up procedures are required, and the lack of chromophore or fluorophore generally necessitates the use of derivatization techniques for their determination by either gas chromatography (GC) or high-performance liquid chromatography (HPLC), unless indirect detection is used. Various methods to analyse glyphosate and AMPA based on GC [6,7], HPLC [8–16] and capillary electrophoresis (CE) [17,18] have been reported. GC approaches have been less favoured since more extensive reactions are needed to convert analytes into volatile compounds. Typical derivatization agents used are trifluoroacetic anhydride in conjunction with trifluoroethanol [6] and 2,2,3,3,4,4,4-heptafluoro-1-butanol [7]. HPLC techniques with the choice of pre- or post-column derivatization, offer more variability. Pre-column procedures have commonly used 9-fluorenylmethyl chloroformate [8,9] with fluorescence detection, although other derivatizing agents such as *p*-toluenesulphonyl chloride [10] and 1-fluoro-2,4-dinitrobenzene [11] can give derivatives absorbing in the UV-visible region. For post-column reaction, *o*-phthalaldehyde and 2-mercaptoethanol [12–14], and ninhydrin [15] are utilised for fluorescence and UV–visible detection respectively. In addition, a post-column indirect detection method has been reported where the background fluorescence is provided by an Al^{3+}–Morin (3,5,7,2′,4′-pentahydroxyl flavone) complex [16]. Comparatively few CE methods are available; one utilises *p*-toluenesulfonyl chloride [17] for derivatization prior to separation, whilst the other incorporates ribonucleotides [18] into the background electrolyte (BGE) to provide the signal for indirect photometric detection. In addition, CE coupled with indirect UV detection has been used to quantify alkylphosphonic acids [19]. Several indirect absorbers in a pH 6.0 borate buffer were investigated, of these phenylphosphoric acid was found to be the most suitable. Following optimization of the electrophoretic parameters, detection limits of less than 0.2 pmol injected were achieved.

The aim of this work was to develop a CE method for the quantification of glyphosate and AMPA at low concentrations, i.e. ng ml^{-1} levels. Indirect detection using a phthalate BGE was chosen to provide mobility and charge matching with glyphosate, and it was shown that field-amplified sample injection can give preconcentration for improving sensitivity by a factor of up to 1000.

2. Experimental

2.1. Chemicals and reagents

The aminomethylphosphonic acid (99%) was provided by the Pesticides Group, (CSL, Slough, UK). N-(Phosphonomethyl)glycine (96%) and potassium hydrogenphthalate (+99%) were obtained from Sigma (Poole, UK), and tetradecyltrimethylammonium bromide (99%) from Aldrich (Gillingham, UK). All other buffer materials and general chemicals were purchased from either Sigma, Aldrich or Fisons (Loughborough, UK). Water purified with an Elgastat UHQII system (Elga, High Wycombe, UK) was used throughout.

2.2. Instrumentation

CE experiments were performed on a Hewlett-Packard ^{3D}CE system (Hewlett-Packard, Cheadle Heath, UK) using HP ChemStations software, with an untreated fused-silica capillary of total length 64.0 cm and effective length 56.0 cm×50 μm I.D.. The capillary was conditioned before use for approximately 1 h using a rinse cycle of 5 min H_2O, 15 min NaOH (1 *M*), 15 min H_2O and 30 min BGE, then electrophoresed for 30 min. Samples were loaded by a 5-s pressure injection (50 mbar) at the cathode and separated under reverse polarity conditions using a voltage of 27.6 kV. The external temperature of the capillary was thermostated at 25°C. The capillary was rinsed prior to each injection with BGE (2 min): for optimisation of pH the BGE was 7.5 m*M*

potassium hydrogen phthalate adjusted with NaOH to cover the pH range 6.0–8.0, and for optimisation of the background absorber concentration 2.5–10 m*M* potassium hydrogen phthalate adjusted to pH 8.0; all contained 0.5 m*M* TTAB as an EOF reverser. Peaks were detected at 240 nm with a bandwidth of 10 nm, using a detector response time of 0.2 s and a 10 Hz data collection rate.

Conductivity measurements were taken with a digital conductivity meter (Philips PW9527, Pye Unicam Limited, Cambridge, UK) and either a 4 cm^3 conductivity cell (cell constant 0.705 cm^{-1} at 20°C) or a 0.25 cm^3 cell (cell constant 1.424 cm^{-1} at 20°C).

2.3. Sample preparation

All standard solutions and BGEs were prepared with purified water (≥18 MΩ cm^{-1}) and filtered through a 0.22 μm filter prior to use. Samples of extracts from spiked wheat were prepared as follows. Milled wheat (20 g) was spiked with the required volume of the standard glyphosate/AMPA solution (5 μg ml^{-1} of each component) then allowed to dry out. Water (40 cm^3) was added, the mixture shaken then blended for ~2 min in a liquidiser. After the solids had settled out, the liquid was decanted off and centrifuged for 5 min at 300 *g*. The supernatant was removed, passed through a 0.2 μm filter then analysed by the developed method using electrokinetic injection since the final concentrations would be too low to permit detection if injected hydrodynamically. Assuming an extraction efficiency of 100%, initial spiking levels were such as to provide concentrations of 0, 25, 100 and 500 ng ml^{-1} of each compound in the final solution.

3. Results and discussion

3.1. Ionization behaviour

pK_a values [20,21] and assignments [20] give the pH-dependent structures of glyphosate shown in Fig. 1. Glyphosate is supplied as the zwitterion; over the pH range 6–10 the dominant form is the species of charge −2, H_2G^{2-}, and the highly ionic nature is evident from the formula with one positively and three negatively charged sites. The ionization behaviour of AMPA is less well characterised, and Fig. 1 shows that there is some discrepancy between the literature pK_a values [22]. Assignments of ionizations are made by analogy with glyphosate [21]. Over the pH range the dominant form is the ion of charge −1.

3.2. Method development

Neither glyphosate nor AMPA was found to have any significant UV absorbance in the wavelength range above 200 nm, thus indirect detection was required. Preliminary studies were carried out with the following BGEs, all containing the cationic detergent tetradecyltrimethylammonium bromide (TTAB), at concentration 0.5 m*M*, as an EOF reverser [23]: 10 m*M* *p*-hydroxybenzoate, 5 m*M*

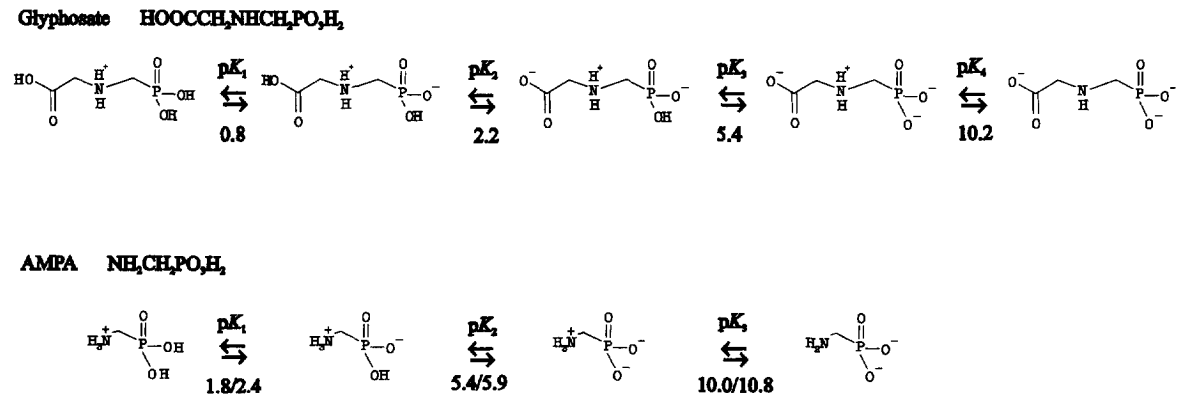

Fig. 1. Ionisation processes for glyphosate and AMPA.

NaCl at pH 5.5; 10 m*M* sodium benzoate, 5 m*M* NaCl at pH 5.6, 5.2 and 6.3

Separations of 0.5 m*M* (84 μg ml^{-1}) and 0.1 m*M* (17 μg ml^{-1}) glyphosate solutions were performed but results were inconsistent; electropherograms showed peaks that could not be attributed to glyphosate alone, and in cases where peaks were not separated alteration of the BGE pH did not increase resolution as expected. Rough calculation of mobilities indicated that glyphosate was likely to be co-migrating with the benzoate system peak. Hence, more mobile indirect absorbers, such as phthalate, pyromellitic acid and chromate, were investigated. Of these, 7.5 m*M* phthalate at pH 7.3 was the most successful and a systematic optimisation of BGE concentration and pH was performed.

The effects of BGE pH and concentration were studied using the conditions described in Section 2. Solutions of both 0.5 and 0.1 m*M* glyphosate were electrophoresed. Resultant electropherograms showed a distinct improvement in the glyphosate peak shape as the pH of the BGE was increased, indicating better mobility matching between the phthalate and glyphosate ions as they became more similarly charged; at pH 6 phthalate and glyphosate have charge ~ -1.8 and -1.5 respectively, whilst at pH 8 they both have charge ~ -2. Peak shapes were assessed in terms of efficiency, *N*, [24] and asymmetry [24], values for the 0.5 m*M* solution being given in Table 1.

It can be seen that peak efficiency rises dramatically above pH 6.0, reaching a likely maximum around pH 6.5–7.0 before decreasing slightly, and is accompanied by a decrease in tailing as indicated by the asymmetry factor. For purposes of optimisation of the background absorber a pH was chosen where peak tailing would be minimal, in this case pH 8.0. Subsequent separations showed that the glyphosate peak shape also improved with increasing phthalate concentration. This resulted in higher peak efficiencies and less peak tailing, as shown in Table 1 for the 0.5 m*M* glyphosate solution. The reason for this improvement is that increases in conductivity of the BGE decreases electromigration dispersion [25], and also causes a greater stacking effect in injection from water.

Since peak efficiency and peak asymmetry started to level off around 10 m*M*, this was decided upon as an optimum concentration since any small gain from using a higher concentration would be offset by increased noise, which is undesirable in indirect detection. Similarly a pH of 7.5 was chosen as a compromise between peak tailing, efficiency and ease of preparation, the pH 8.0 BGE being extremely slow to stabilise at that pH. With this BGE, i.e. 10.0 m*M* potassium hydrogenphthalate adjusted to pH 7.5 with NaOH and containing 0.5 m*M* TTAB, a dynamic reserve, *DR*, (signal/peak-to-peak noise on top of the signal) of 2200 was achieved. Under these conditions glyphosate and AMPA migrated at around 3.1 and 3.5 min respectively, see Fig. 2. Sensitivity towards AMPA is lower than that for glyphosate since, owing to its lower charge at that pH, it displaces less of the phthalate.

Table 1
Effects of pH and BGE concentration upon glyphosate peak shape and efficiency

Phthalate concentration (m*M*)	pH	Peak asymmetry factor	Peak efficiency, $N/10^5$ theoretical plates
7.5	6.0	4.3	0.25
7.5	6.5	2.3	3.3
7.5	7.0	1.7	3.3
7.5	7.5	1.1	2.9
7.5	8.0	1.0	2.8
2.5	8.0	4.8	0.5
5.0	8.0	3.7	1.1
7.5	8.0	2.7	2.9
10.0	8.0	1.3	3.8
12.5	8.0	1.0	3.9

Conditions are as described in Section 2.

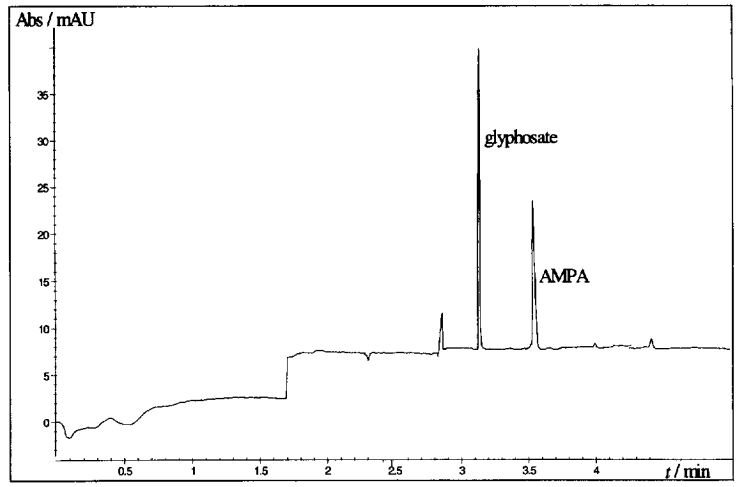

Fig. 2. Separation of an equimolar mixture of glyphosate and AMPA using the developed method. Conditions: capillary 64.5 cm (56.0 cm to detector)×50 μm I.D.; temperature 25°C; BGE 10.0 m*M* potassium hydrogen phthalate adjusted to pH 7.5 with NaOH, 0.5 m*M* TTAB; injection 5 s at 50 mbar pressure (6.7 nl); applied voltage 27.6 kV, reverse polarity; detection $\lambda = 240$ nm (sample), 350 nm (reference). Analyte: sample solution containing 1.0 m*M* glyphosate and 1.0 m*M* AMPA in water.

3.3. Method assessment

The finalised conditions are summarised in the legend to Fig. 2 with other conditions (rinse protocol, detector bandwidth, response time and collection rate) as in Section 2. The developed method was then assessed in terms of precision and linearity for both glyphosate and AMPA in separate experiments. Limits of detection were subsequently determined.

3.3.1. Precision

The repeatability was assessed by ten replicate injections of 0.5 m*M* (84 μg ml^{-1}) and 0.1 m*M* (17 μg ml^{-1}) solutions for glyphosate, and 0.1 m*M* (11 μg ml^{-1}) for AMPA. Resultant peak areas were normalised to allow for differential migration rates [26], the results being tabulated in Table 2.

From the coefficients of variation (% R.S.D. values) it is clear that both migration times and normalised peak areas are quite repeatable. The R.S.D. values of 1–2% for normalised peak areas are better than those typically found, R.S.D. values of 5% for peak areas being common in indirect detection [27,28]. Reasons for the good R.S.D. values observed here could include the excellent dynamic reserve, discussed in Section 3.2, and the fact that the optimised conditions use a pH where charges on both analytes and the BGE absorber are invariant to fluctuations in the BGE pH. Local fluctuations in the capillary, affecting for example temperature and concentrations, have been shown to be generally more important than detector signal to noise characteristics in determining precision in indirect detection [29].

3.3.2. Linearity

Calibrations were performed over the concentration ranges 0.01–1.00 m*M* (1.7–170 μg ml^{-1}) for glyphosate and 0.01–1.00 m*M* (1.1–110 μg ml^{-1}) for AMPA. The relationship between concentration and response, i.e. normalised peak area, was found to be linear for both compounds over the ranges studied, the regression lines having the following equations; slopes and intercepts are given with 95% confidence limits.

Glyphosate $y = (13.2 \pm 0.2) \cdot 10^{-2} x + (7.9 \pm 10.9) \cdot 10^{-4}$
correlation coefficient, $r = 0.9998$
AMPA $y = (10.0 \pm 0.1) \cdot 10^{-2} x + (6.3 \pm 6.0) \cdot 10^{-4}$
correlation coefficient, $r = 0.9999$

The linear range is at least two orders of mag-

Table 2
Repeatability data for glyphosate and AMPA

Replicate	0.5 m*M* Glyphosate		0.1 m*M* Glyphosate		0.1 m*M* AMPA	
	t_{mig} (s)	Peak area/t (mAU)	t_{mig} (s)	Peak area/t (mAU)	t_{mig} (s)	Peak area/t (mAU)
1	190.2	0.0696	186.6	0.0128	204.6	0.0102
2	189.6	0.0707	185.4	0.0127	204.0	0.0102
3	189.0	0.0711	185.4	0.0131	204.0	0.0098
4	188.4	0.0710	184.8	0.0133	203.4	0.0103
5	187.8	0.0710	184.2	0.0133	203.4	0.0101
6	187.2	0.0712	183.6	0.0134	202.8	0.0103
7	187.2	0.0713	183.6	0.0136	202.8	0.0101
8	187.2	0.0718	183.6	0.0133	202.2	0.0102
9	187.2	0.0720	183.0	0.0131	202.2	0.0101
10	186.6	0.0725	183.0	0.0132	201.6	0.0102
Average	188.0	0.0712	184.3	0.0132	203.1	0.0102
$\sigma_n - 1$	1.2	$7.9 \cdot 10^{-4}$	1.2	$2.7 \cdot 10^{-4}$	0.95	$1.4 \cdot 10^{-4}$
% R.S.D.	0.6	1.1	0.6	2.0	0.5	1.4

Conditions are as summarised in the legend to Fig. 2.

nitude and a separate calibration over the range 0.01 – 5 m*M* showed the linear range to be approaching three orders of magnitude. This is exceptionally good for indirect detection. The technique suffers from having a poor linear dynamic range, normally at best two orders of magnitude for absorbance [30].

3.3.3. Limit of detection and transfer ratio

The concentration limit of detection (C_{LOD}), defined using the convention of a peak height equal to twice peak-to-peak noise [31], was determined from measurements with 0.05 m*M* standard solutions (6.7 nl injection) to be for glyphosate 5 μM, i.e. 0.8 μg ml^{-1}, and for AMPA 5 μM, i.e. 0.6 μg ml^{-1}. Since peak-to-peak noise is generally considered as 5–6 times greater than root-mean-square (RMS) noise, an alternative definition of C_{LOD} as three times RMS noise [32] would give a limit of detection of ~1.5 μM (0.2 μg ml^{-1}) for glyphosate. Measurements with 5 μM glyphosate showed that the first convention, giving LOD of 5 μM, is operationally most realistic in terms of reliable peak detection under the CE conditions used.

Peak widths (full width at base) were found to be 5.2 mm (glyphosate) and 7.9 mm (AMPA). These may be compared with the calculated injection plug length of 3.4 mm for the 6.7 nl pressure injection used, and indicate that despite compression of the analyte zone due to stacking on injection from water, diffusion still causes significant band broadening.

The concentration limit of detection (C_{lim}) at the detector, not taking into account stacking and dilution on capillary due to diffusion etc., is normally calculated by the equation;

$$C_{lim} = C_m/(TR \cdot DR) \quad (1)$$

where C_m is the concentration of UV absorbing component in the BGE, TR is the transfer ratio and DR is the dynamic reserve [33]. Using the convention of calculating the LOD on the basis of a peak height equal to twice peak-to-peak noise, Eq. 1 becomes;

$$C_{LOD} = C_m/(TR \cdot DR/2) \quad (2)$$

The transfer ratio is defined as the number of indirect absorbing ions displaced by one analyte ion, and has been measured for both glyphosate and AMPA according to the procedure described by Williams et al. [34]. This gives TR values of 0.97 and 0.75 for glyphosate and AMPA respectively. Similarly, values of 0.98 and 0.75 have been calculated from the slopes of the calibration lines; slope multiplied by the internal volume of the capillary and then divided by the volume of analyte injected and response of the BGE absorber. Using these, values for C_{LOD} of 9 μM (1.6 μg ml^{-1}) glyphosate and 12 μM (1.3 μg

ml^{-1}) AMPA were obtained, these being approximately twice those found experimentally under stacking conditions. Theoretical *TR* values of 1.07 and 0.90 were predicted for glyphosate and AMPA respectively, using the approach of Poppe [35]. Unlike the simplest theory [33] which assumes transfer ratios to be determined by 1:1 charge displacement, this treatment involves coupled transport equations for all ions which are solved as part of a computer program. Theoretical values are approximately 10–15% higher than those found experimentally, which is good agreement considering that the model does not take into account any deviations from ideality in ionic equilibria and transport.

3.4. Preconcentration

In hydrodynamic injection, the quantity loaded is nearly independent of the sample matrix whilst the volume loaded depends on the capillary dimensions, BGE and sample viscosity, applied pressure and time. In electrokinetic injection, the amount loaded is dependent on the EOF, the conductivities of the BGE and sample solution, and the electrophoretic mobilities of the analytes. An injection discrimination exists with the more mobile species being loaded to a greater extent. The majority of the work was therefore carried out with glyphosate alone to avoid any injection bias and possible misinterpretation of results.

Stacking and field-amplified sample injection (FASI) [36] arise from field strength differences between the sample zone and BGE. For stacking to occur, samples must be dissolved in water or low conductivity BGE. Previous theoretical and experimental measurements using hydrodynamic injection under gravity, suggested that optimal stacking is obtained when the sample solution contains 10% BGE [37]. The principles of stacking are much the same for both injection techniques, but on electrokinetic injection an enhanced field strength will exist at the point of injection, thus allowing a greater number of appropriately charged ions to be loaded. Manipulation of the capillary during the injection process, e.g. when switching the capillary from the BGE vial to the sample vial, can result in the BGE boundary at the end of the capillary being disturbed and consequently the electric field at the injection point may not be amplified properly. By introducing a short plug of water or low conductivity electrolyte into the capillary prior to sample injection, a high electric field strength is established from the beginning of the injection and ensures proper field amplification. Enhancements of several hundred-fold in the amount injected have been reported using this method, with no loss in resolution [36].

3.4.1. Effect of the percentage of BGE in sample diluent

In this study, we have investigated optimisation of stacking conditions in both pressure and electrokinetic injection with the goal of leading to lower detection limits. The effect of the percentage of BGE in the sample diluent was studied using 0.5 m*M* (84 $\mu g\ ml^{-1}$) glyphosate standards prepared in sample diluents containing 0–95% BGE. Samples were injected by both pressure and electrokinetic means; a voltage of −6.3 kV applied for 5 s was calculated to load an amount of glyphosate (under non-stacking conditions) equivalent to that loaded by 5 s at 50 mbar pressure. The effect of % BGE in sample diluent upon the separation is shown in Table 3 for both injection modes. Peaks were assessed in terms of peak height and normalised peak area.

Table 3 shows peak height decreasing as the proportion of BGE in the sample solution is increased, corresponding to a decline in stacking as the sample conductivity rises. This trend is most significant for the electrokinetic injection where increased stacking results in an increase in the amount injected, as shown by the normalised peak areas. Electrokinetic stacking from water was found to load at least ten times more than that from BGE, i.e. non-stacking conditions. However, the peak shapes were found to indicate severe overloading, and to be of little analytical use; ~60% BGE being necessary to produce a reasonable peak shape. For pressure injection, Table 3 shows that the normalised peak area remained the same, as expected, with peak heights indicating that injection from water is necessary for maximum stacking and therefore sensitivity. The improvement in peak width is shown in Fig. 3.

The procedure was repeated using a 0.05 m*M* (8 $\mu g\ ml^{-1}$) glyphosate standard with electrokinetic injection only, but once again overloaded peaks were obtained when stacking from water, and the largest

Table 3
Effect of % BGE in sample solution upon the peak heights and normalised peak areas obtained on separation of 0.5 m*M* glyphosate.

%BGE in sample solution	Response (pressure injection)		Response (electrokinetic injection)	
	Peak height (mAU)	Peak area/*t* (mAU)	Peak height (mAU)	Peak area/*t* (mAU)
0	24.0	0.0744	73.6	0.8079
10	20.0	0.0725	31.3	0.2166
20	21.2	0.0736	29.7	0.1613
40	17.4	0.0748	18.8	0.0990
60	13.9	0.0730	13.9	0.0746
80	11.6	0.0744	11.1	0.0629
90	–	–	9.6	0.0577
95	10.0	0.0723	–	–

Conditions: pressure injection, as for Fig. 2, analyte 0.5 m*M* glyphosate in 0–95% BGE; electrokinetic injection, as for Fig. 2, injection 5 s at −6.3 kV, analyte 0.5 m*M* glyphosate in 0–90% BGE.

decrease in response was observed when changing from water to the 10% BGE solution. Further studies using sample solutions containing 0–10% BGE indicated that 1% BGE would be sufficient to reduce response by at least 50%, thus implying that any contamination of an aqueous solution (0% BGE) could result in similar losses. A particular area of concern was possible contamination caused by the capillary as it moved from one vial to the next. By incorporating a water injection (5 s at 50 mbar pressure) into the method, prior to the sample injection but after the BGE rinse, any remaining BGE on the outside walls of the capillary should be removed and the precision of the method improved. This was found to be the case. For a series of ten replicate injections, each set from a separate vial of 0.01 m*M* (1.7 μg ml^{-1}) glyphosate, results obtained for (peak area/*t*)/mAU (i) without and (ii) with pre-injection of water were: (i) 0.096±0.020 (20% R.S.D.); (ii) 0.131±0.011 (8% R.S.D.). Both the precision and the amount loaded are seen to improve when a pre-injection plug of water is used, part of this effect being due to field-amplification on sample injection.

Subsequent experimental work involving an even lower concentration of glyphosate, 5 m*M* (0.8 μg ml^{-1}) in 0–90% BGE, made use of this revised injection procedure to determine if contamination

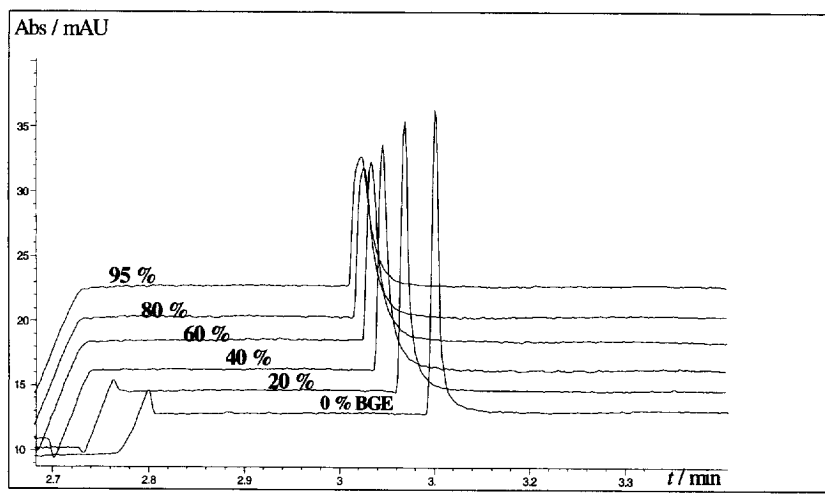

Fig. 3. Effect of BGE content of sample solution upon sample stacking for pressure injection. Conditions: as for Fig. 2. Analyte: 0.5 m*M* glyphosate in 0–95% BGE.

was the major cause of the reduced response previously observed. Although conductivity increased linearly with BGE content of the sample solution, the corresponding decrease in observed response was non-linear, as shown in Fig. 4. Peak height decreased similarly and was accompanied by a slight increase in half-height width, $W_{0.5}$, signifying a loss in peak definition. The most significant decrease in response, ~60% less than that obtained by stacking from water, occurred with 1% BGE in the sample solution. This implies that conductivity of the sample solution was the major concern at this level of glyphosate, and not cross-contamination by the capillary. However, at even lower concentrations of glyphosate it is likely that cross-contamination would become more important.

From this work it is evident that injection from water is necessary for optimum stacking. This does not affect the LOD reported in Section 3.3 for glyphosate by pressure injection, namely 5 μM (0.8 μg ml^{-1}). However, with electrokinetic injection the size of the signal is such that detection of a concentration of 0.1 μM (17 ng ml^{-1}) glyphosate should also be possible using the same conditions. Use of organic solvent additions has been shown to improve sensitivity and stacking [38,39]. Attempts to utilise this strategy by incorporating methanol into the sample solution, to reduce the conductivity and give a greater field strength on injection, were largely unsuccessful; using 10% methanol in the glyphosate solution gave variable results, some favourable, others not. It was therefore concluded that no obvious benefit was to be gained with this approach.

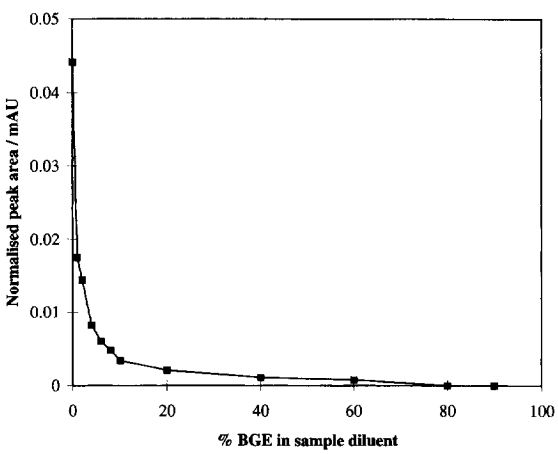

Fig. 4. Decreasing response observed for electrokinetic injection of 0.005 mM glyphosate as BGE content of sample solution increased. Conditions: as for Fig. 2, electrokinetic injection 5 s at −6.3 kV. Analyte: 0.005 mM glyphosate in 0–90% BGE.

3.4.2. Size of water plug for FASI

As discussed in the previous section, pre-injection of a water plug has beneficial effects. A systematic study was therefore carried out on the effect of variation of the length of water plug. This must be of sufficient length to ensure field amplification but not so long that peak broadening, as a result of pressure-driven flow, occurs. Using the general CE conditions but with a plug of water, varying in size from 0–10 s of 50 mbar applied pressure, being introduced prior to sample injection, an aqueous solution of 5 μM (0.8 μg ml^{-1}) glyphosate was electrophoresed. Results for peak heights, $W_{0.5}$ values and normalised peak areas showed that the most signal enhancement was obtained over the plug size range of 1–8 s. Increases were observed for all three parameters over those obtained for conventional electrostacking from water, for example a 50% increase in peak area/t from 0.028 to 0.041 mAU. Values of all parameters remained relatively constant over the 1–8 s range before showing a noticeable decline with a 10 s plug size. A 2 s plug size was subsequently selected for future work although longer times ($\leq$8 s) would have been just as suitable. Excellent agreement is seen between data obtained here and previously in Section 3.4, with normalised peak area for a 5 s electrokinetic injection of 5 μM glyphosate without BGE in the sample solution equal to 0.04 mAU in both studies.

3.4.3. Sample injection time

Chien and Burgi [37] have reported that even larger enhancements in signal detectability can be achieved by using longer injection times, since peak widths in field-amplified injection do not increase as fast as those obtained via conventional electro-injection. Injection times were varied over the range 5–60 s at −6.3 kV for 5 μM (0.8 μg ml^{-1}) and 0.5 μM (84 ng ml^{-1}) glyphosate solutions, and resultant peaks assessed in terms of peak height, $W_{0.5}$ and normalised peak area. For the 5 μM glyphosate solution, all three peak parameters increased steadily

but peaks became overloaded with injection times >10 s. For the 0.5 μM solution, peak height and area values showed a general increase whilst $W_{0.5}$ remained effectively constant (1.0±0.1 s) up to a 45 s injection time. At an injection time of 60 s, a significant increase in $W_{0.5}$ to 1.5 s indicated that 45 s was the upper limit for injection time without compromising stacking performance.

Fig. 5 gives a graphical representation of response (normalised peak area) as a function of sample injection times. It can be seen that a non-linear relationship exists between the two variables at the higher concentration. This was also found to be the case at the lower concentration, when comparing correlation coefficients obtained for fitting linear and quadratic equations to the lines:

Linear	$r=0.996$ (5 μM),	$r=0.992$ (0.5 μM);
Quadratic	$r=1.000$ (5 μM),	$r=0.997$ (0.5 μM)

Having ascertained that injection times up to 45 s could be utilised with no apparent detrimental effects upon the separation, a solution of 0.01 μM (2 ng ml^{-1}) glyphosate was analysed using 15–45 s injection times. Detection at this level can be observed in Fig. 6. No attempts were made to detect even lower concentrations since trace contamination was becoming more of a problem.

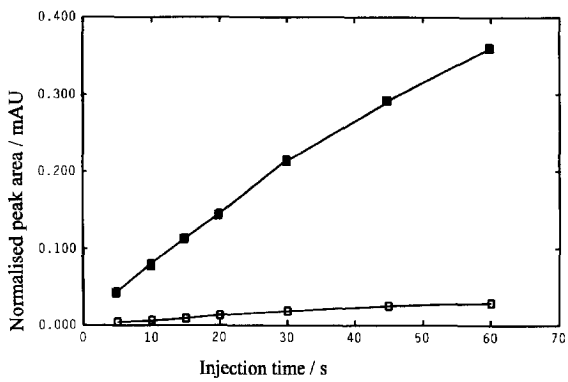

Fig. 5. Variation of observed response with sample injection time for electrokinetic injection of 5 μM (■) and 0.5 μM (□) glyphosate in water. Conditions: as for Fig. 2, electrokinetic injection 5–60 s at −6.3 kV.

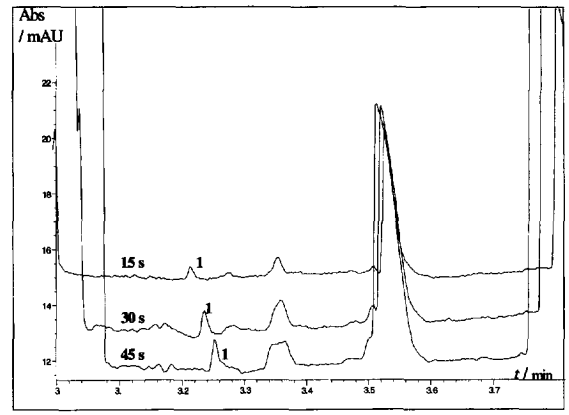

Fig. 6. Detection of 0.01 μM glyphosate (1) in water using FASI with long injection times. Conditions: as for Fig. 2. Injection sequence; H_2O, 2 s at 50 mbar pressure, then sample solution, 15–60 s at −6.3 kV electrokinetic injection.

3.5. Analysis of wheat samples

Several reports [8,13,15] have detailed the extraction of glyphosate and AMPA from a variety of animal, soil, water and plant matrices, the latter being of particular interest here. Preliminary CE separations were carried out with the intention of discovering whether real samples could be analysed successfully by the developed method following a simple extraction step, but without any further pretreatment. The extraction procedure is detailed in Section 2. Glyphosate and AMPA peaks could be identified using spiking and mobility data obtained from the electrophoresis of standard solutions of glyphosate (5 μM) and AMPA (5 μM) under the same conditions; for glyphosate, $\mu=4.7\cdot10^{-8}$ m^2 V^{-1} s^{-1}, and for AMPA $\mu=4.1\cdot10^{-8}$ m^2 V^{-1} s^{-1}, where μ is the observed electrophoretic mobility.

No evidence for glyphosate or AMPA was found in any of the spiked wheat samples, all electropherograms looking identical to that of the blank sample. Further increases in sample injection time resulted in considerable shifts in the migration times of the peaks, but no detection of either analyte. Suspecting that the sample matrix was the cause of the problem, the blank wheat extract was spiked at the 500 ng ml^{-1} level and re-analysed. Once again

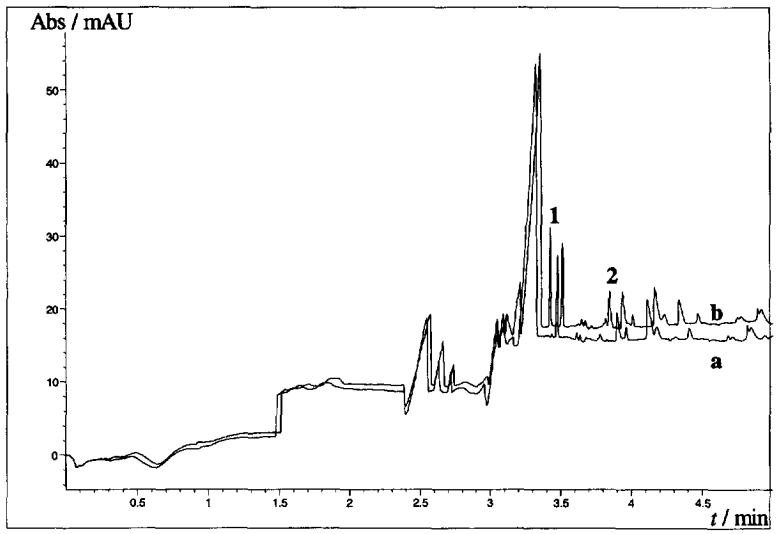

Fig. 7. Identification of glyphosate (1) and AMPA (2) peak positions in an electropherogram of a wheat extract sample using a pre-injection of a mixture containing glyphosate and AMPA in water, each at the 500 ng ml^{-1} level. Conditions: as for Fig. 2. Injection procedure (a) wheat extract; H_2O, 2 s at 50 mbar pressure, then sample solution 5 s at −6.3 kV; (b) wheat extract with pre-injection of standard; H_2O, 2 s at 50 mbar pressure, then glyphosate–AMPA mixture (each 500 ng ml^{-1}), 5 s at −6.3 kV, followed by sample, 5 s at −6.3 kV.

no peaks were observed with electrokinetic injection. Further sample injections were made in conjunction with pre- and post-injections of the 500 ng ml^{-1} mix. Comparing electropherograms of the spiked 'blank' wheat extract with that for the same sample and a pre-injection of the standard mix (Fig. 7), only the latter showed evidence of glyphosate and AMPA. The pattern of peaks in the wheat sample extracts was very reproducible and the positions of the glyphosate and AMPA peaks should allow resolution from other matrix components. The conductivity of the wheat extract was subsequently measured and found to be around 3 mS cm^{-1}, approximately 1000 times greater than a 5 μM solution of glyphosate in water, thus confirming that the sample matrix was inhibiting electrokinetic injection.

4. Conclusions

A CE method has been developed for the determination of glyphosate and AMPA in pure water. Using a BGE solution with TTAB as EOF reverser and indirect detection with phthalate, which has charge and mobility at pH 7.5 well matched to that of glyphosate, analysis times are less than 4 min and excellent values are found for the precision and dynamic range of the method. Transfer ratios for both analytes determined experimentally from observed signal responses are in good agreement with values calculated theoretically. Detection limits using hydrodynamic injection were ~10 μM (1–2 μg ml^{-1}) and 5 μM (0.5–1 μg ml^{-1}) for conditions of no stacking and stacking respectively, whilst 0.1 μM (20 ng ml^{-1}) was achievable with electrokinetic injection from water. With field amplification 0.01 μM (2 ng ml^{-1}) could be detected, this representing a signal enhancement of ~1000 over pressure injection from BGE, i.e. no stacking.

Pre- or post-injection of 500 ng ml^{-1} analyte mixtures with wheat sample extracts showed that glyphosate and AMPA peaks were resolved from those of other matrix components. However, the high conductivity of the extracts was found to inhibit electrokinetic injection of the analyte directly from spiked samples, and hydrodynamic injection, while

possible, did not have the sensitivity necessary to detect glyphosate and AMPA at the ng ml^{-1} levels required. CE cannot, therefore, offer a rapid alternative to other methods (GC and LC) which require extensive pretreatment and derivatization steps. Clean-up involving a reduction in the conductivity of sample matrices will be necessary if the method is to be successfully applied to real samples.

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