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# Separation of Maillard reaction products from xylose–glycine and glucose–glycine model systems by capillary electrophoresis and comparison to reverse phase HPLC

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Capillary electrophoresis (CE) and reverse phase HPLC were used to analyse Maillard reaction products (MRPs) formed in refluxed, aqueous xylose–glycine and glucose–glycine model systems. CE was shown to resolve many more components than reverse phase HPLC. Ultrafiltration was used to separate the MRPs into three molecular weight fractions, nominally > 3000, between 3000 and 1000, and < 1000 daltons. Components of the lowest molecular weight fraction migrated as sharp, well-resolved peaks by CE, whereas the components of the higher molecular weight fractions (melanoidin) migrated as a single broad peak in each case. The melanoidin and the majority of the low molecular weight compounds migrate as anions in borate buffer at pH 9.3, some of this anionic character being due to complexation with borate. The majority of colour in the systems was attributed to the melanoidin, with only two other CE peaks in the xylose–glycine, and one in the glucose–glycine systems having significant absorbancies in the visible region. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The Maillard reaction is responsible for much of the colour which develops in many foods on thermal processing. It results in the formation of complex mixtures of coloured and colourless Maillard reaction products (MRPs) which possess a wide range of polarities and molecular weights, making their analysis difficult. The most common analytical method used is reverse phase HPLC (Miksik *et al.*, 1990; Porretta, 1992; Bailey *et al.*, 1996), where separation of some of the lower molecular weight products, such as 5-hydroxymethylfurfural (HMF), has been achieved. However, the higher molecular weight melanoidins have proved even more difficult to analyse, eluting as unresolved bands or humps with reverse phase HPLC (Tomlinson *et al.*, 1994; Bailey *et al.*, 1996).

Recently, the technique of capillary electrophoresis (CE) has found a place in food analysis (Lindeberg, 1996). Since melanoidins have been shown to be amphoteric in character in work by Gomyo and Horikoshi (1976) and O'Reilly (1983) using isoelectric focusing, then CE could be a useful technique for their analysis. Reports on the application of CE to the separation of MRPs (Deyl *et al.*, 1990; Tomlinson *et al.*, 1993, 1994; Ames *et al.*, 1997) have shown that CE resolves more components than reverse phase HPLC. Deyl *et al.* (1990) used CE to investigate the reaction products formed from glucose or ribose with either glycine, alanine or isoleucine, before and after derivatisation with 2,4-dinitrophenylhydrazine or phenyl isothiocyanate. Tomlinson *et al.* 1993, 1994) compared the techniques of reverse phase HPLC and CE for the separation of MRPs from spray-dried glucose–glycine and HMF–glycine model systems. Ames *et al.* (1997) applied CE to the separation of methanol-extractable components of a starch–glucose–lysine model food system.

This paper (a) compares reverse phase HPLC and CE for their abilities to separate the reaction products of two model Maillard systems, (b) describes the CE separation profiles obtained for different molecular weight fractions of the reaction products and, (c) compares the CE separation achieved using different buffers.

## MATERIALS AND METHODS

### Reagents

Xylose and glycine (99+% grade) and glucose (ACS grade) were obtained from Aldrich (Gillingham, UK). High purity water was prepared in-house from a Purite

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Labwater RO50 unit (High Wycombe, UK). Methanol was HPLC grade from Rathburn (Walkerburn, UK). Sodium hydroxide was GPR grade from BDH (Lutterworth, UK). Sodium hydroxide solutions for CE were obtained from Fluka (Gillingham, UK). Buffers used for CE were 50 mM tetraborate pH 9.3 (pre-prepared CZE buffer, Hewlett Packard, Bracknell, UK); 100 mM disodium orthophosphate (Aldrich) adjusted to pH 9.3 with NaOH, and 100 mM sodium tetraborate (Aldrich) adjusted to pH 9.3 with HCl. The latter two buffers were diluted with water to give a range of concentrations.

### Model system for the preparation of Maillard reaction products

Solutions of glycine (7.5 g; 0.1 mol) and xylose (15 g; 0.1 mol) or glucose (18 g; 0.1 mol) in water (100 ml), were heated under reflux for 2 h. The pH of the reaction mixture was measured throughout heating using an autoclavable ACWL/A-300 Russell electrode (Auchtermuchty, UK) and maintained at pH 5 by the automatic addition of 3 M NaOH by means of a Hanna HI 8710E pH controller and a peristaltic pump (Orme Scientific Equipment, Manchester, UK).

Samples were removed for both CE and HPLC analysis after 0, 10, 30, 60, 90 and 120 min and immediately cooled on ice (Bailey *et al.*, 1996).

### Isolation of high molecular weight Maillard reaction products

An Amicon (Stonehouse, UK) ultrafiltration stirred cell fitted with either a 3000 or a 1000 dalton molecular weight cut-off ultrafiltration membrane, operated under 3.5 bar nitrogen pressure, was used to isolate the high molecular weight MRPs. The molecular weight cut-off point for the ultrafiltration membranes is based on glomerular protein size, so, for their use with melanoidins, these are nominal molecular weight cut-off points.

A 20 ml sample of the amino acid–sugar mixture, which had been refluxed for 2 hours, was prefiltered with a 0.2 $\mu$m PVDF filter (Whatman, Maidstone, UK) before ultrafiltration using a 3000 molecular weight cut-off membrane. This 20 ml sample was reduced under pressure to 1 ml with the filtrate being collected for the next ultrafiltration stage. The retained 1 ml fraction was diluted with 10 ml of water and re-concentrated to 1 ml. This washing procedure was repeated once more to give a sample comprising of nominally 99.95% of material with a molecular weight of above 3000 daltons.

The first ultrafiltrate with a molecular weight of below 3000 daltons, was treated in the same way as above, but using a 1000 molecular weight cut-off membrane. This gave a 1 ml sample comprising of nominally 99.95% of material with a molecular weight between 1000 and 3000. The ultrafiltrate with a molecular weight of less than 1000 was also retained for analysis.

### Capillary electrophoresis (CE)

All CE experiments were performed using an Hewlett-Packard$^{3D}$ Capillary Electrophoresis system equipped with built-in diode-array detector and HP ChemStation for system control, data collection and data analysis (Hewlett-Packard, Bracknell, UK). An uncoated fused silica capillary of 50 $\mu$m internal diameter, 485 mm total length and 400 mm to the detector (Hewlett-Packard) was used throughout. New capillaries were conditioned before use by flushing with 1 M NaOH (30 min), 0.1 M NaOH (20 min), water (15 min) and running buffer (5 min). Between runs, capillaries were flushed with 0.1 M NaOH, followed by running buffer, each for 3 min.

All samples were diluted ten-fold with water and passed through a 0.2 $\mu$m PVDF filter before injection onto the capillary. Samples were applied to the anionic end of the capillary using pressure injection (5 s at 50 mbar). The capillary temperature was maintained at 25°C and the maximum threshold value for the current was set at 100 $\mu$A. Separation was achieved by application of a voltage of 25 kV for 15 min, or 15 kV for 60 min for the higher buffer concentrations. Electrode buffers were renewed every three runs. Electropherograms were monitored at 200, 280, 254, 360 and 460 nm, with raw spectral data collection between 190 and 600 nm.

### HPLC

A Hewlett-Packard 1050 quaternary pump, autosampler and diode-array detector equipped with HP ChemStation for data analysis, were used throughout. This was fitted with a reverse phase 250 mm, 4.9 mm i.d. column, packed with Spherisorb ODS2, particle size 5 $\mu$m (Hichrom, Theale, UK) connected to an ODS2 guard column (Hichrom). A linear methanol/water gradient of 5–45% methanol was applied over 30 min. The column was washed between runs with 100% methanol (Bailey *et al.*, 1996).

Samples (20 $\mu$l) were applied to the column with no pre-treatment. Chromatograms were monitored at 254, 280, 360 and 460 nm, with raw spectral data collection between 190 and 600 nm.

## RESULTS AND DISCUSSION

### Comparison of HPLC and CE

When the xylose–glycine and glucose–glycine 2 h reaction mixtures were examined by reverse phase HPLC, few peaks were resolved at any of the wavelengths measured, the majority of components being unretained by the column. Most information was obtained from the 254 nm traces which are shown in Fig. 1 (it is impractical to monitor peaks at 200 nm with reverse

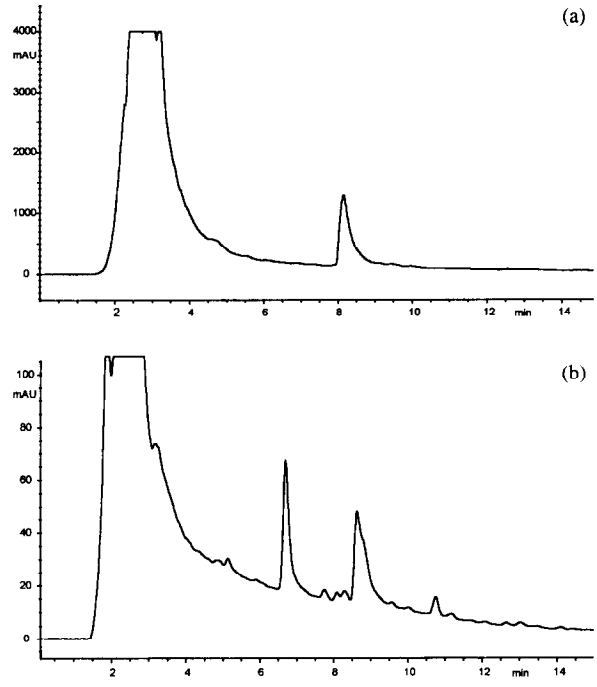


**Fig. 1.** HPLC chromatograms from (a) xylose–glycine and (b) glucose–glycine model systems after refluxing for 2 hours at pH 5. Absorbance at 254 nm.

phase HPLC when gradient elution is involved, due to absorbance by organic solvents at this wavelength). However, when CE was used to examine the same two systems with monitoring at 200 nm (Figs 2f and 3f), there was a dramatic increase in resolution compared to HPLC. Figures 2 and 3 follow the development of MRPs in the two systems over the 2-h heating time. The complexity of the electropherograms increased over time as the number of MRPs increased and, in the xylose–glycine system, over 30 peaks are resolved in the 2-h reaction mixture. Glucose is known to react more slowly than xylose in the Maillard reaction and it produced a much less brown solution after 2 h reaction with glycine. Figure 3 shows that the MRPs are developing more slowly in this system than in the xylose–glycine system (Fig. 2). After 2 h, the glucose–glycine system showed a similar degree of complexity to the xylose–glycine system after 30–60 min. A wavelength of 200 nm showed the largest number of resolved peaks on the electropherograms and all peaks detected at higher wavelengths were also seen at 200 nm.

The diode array data showed that the broad hump which developed over time in Figs 2 and 3 was responsible for most of the colour in the reaction mixtures. However, spectral analysis of individual peaks shows that the peaks at 4.5 and 4.8 min in the xylose–glycine system, and at 4.4 min in the glucose–glycine system, have significant absorbance in the visible region and therefore also contribute to the colour of the respective systems.

Previous work with CE on similar Maillard model systems by Deyl *et al.* (1990) gave less complicated

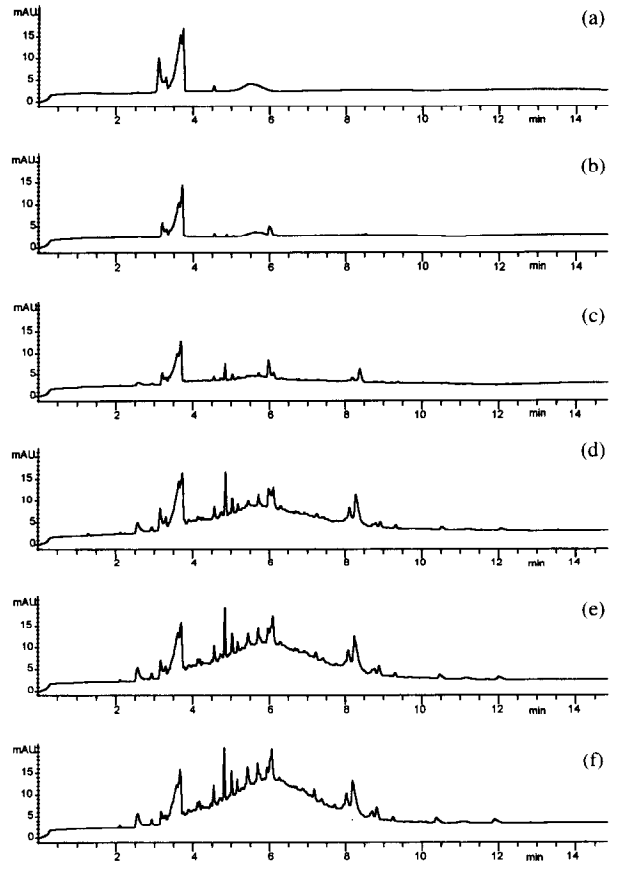


**Fig. 2.** Electropherogram of xylose–glycine model system. 50 mM borate buffer pH 9.3, 25 kV. Time of reaction (a) 0, (b) 10, (c) 30, (d) 60, (e) 90, and (f) 120 min. Absorbance at 200 nm.

electropherograms with only 5–7 peaks being seen. However, Deyl *et al.* (1990) used electrokinetic sample loading, which only allows cations to load onto the capillary, any neutral or negatively charged compounds staying in the sample vial. Electropherograms of similar complexity to those seen in the current study were observed by Tomlinson *et al.* (1994) with phosphate buffer at pH 6.5, and Ames *et al.* (1997) with borate buffer at pH 9, where pressure was used to load the total sample.

## CE of high and low molecular weight Maillard reaction products

Ultrafiltration was used to separate MRPs from the xylose–glycine system into three fractions of different molecular weight ranges, i.e. over 3000, between 1000 and 3000, and below 1000 daltons. The two higher molecular weight fractions, consisting of melanoidins, migrated as broad humps (Fig. 4a and b). The fraction with a nominal molecular weight above 3000 daltons gave a hump with the same mean migration time as the 1000–3000 daltons fraction, but which was less broad. The broadness of these peaks could be due to a wide range of components with closely related molecular

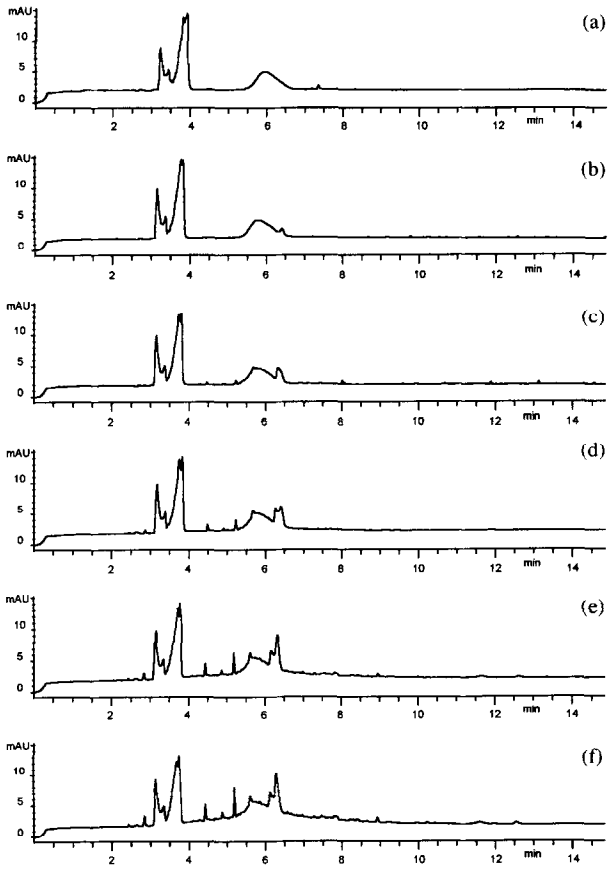


**Fig. 3.** Electropherogram of glucose–glycine model system. 50 mM borate buffer pH 9.3, 25 kV. Time of reaction (a) 0, (b) 10, (c) 30, (d) 60, (e) 90, and (f) 120 min. Absorbance at 200 nm.

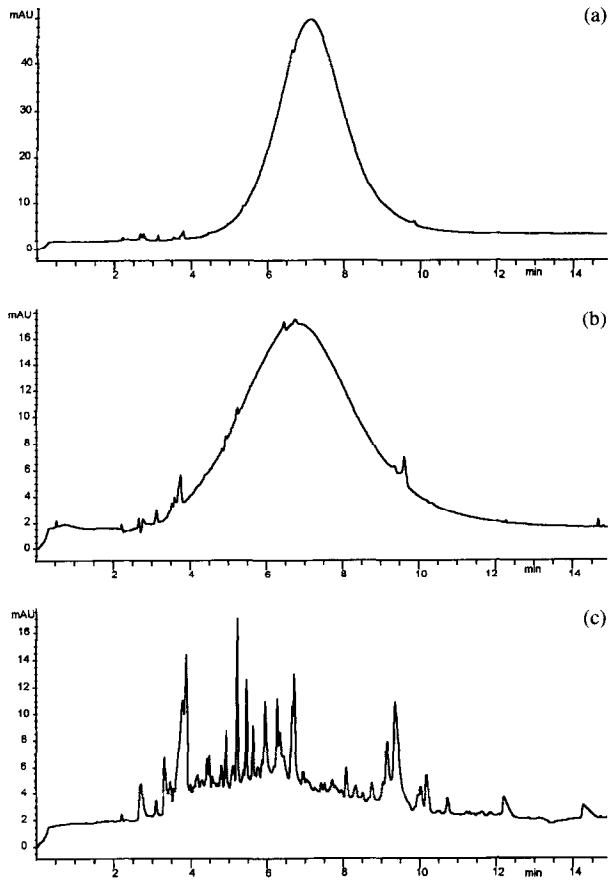


**Fig. 4.** Electropherograms of ultrafiltration fractions from xylose–glycine model system after 2 hours under reflux at pH 5. 50 mM borate buffer pH 9.3, 25 kV. Absorbance at 200 nm. (a) above 3000, (b) between 1000 and 3000 and (c) below 1000 daltons molecular weight.

weight to charge ratios, the range of molecular weight to charge ratio being larger in the 1000–3000 than in the 3000 dalton fraction. In CE, separation of species is achieved by differences in mobility in an electrical field, this in turn being dependent on the charge of the species and its size. In the case of polymers of repeating units, the charge to mass ratio would not be expected to change much with the size of the polymer, thus polymers of varying sizes will effectively co-migrate, giving a broad band. The broadness of the melanoidin peaks could be explained by them being of a polymeric nature. In Fig. 4c, the low molecular weight MRPs approach baseline resolution, with more peaks discernible, compared to the electropherogram obtained before the removal of the higher molecular weight melanoidins (Fig. 2f).

### Effect of buffer on CE separation of MRPs

During analysis by CE, neutral complexes migrate with the electro-osmotic flow (EOF), while cations and anions have migration times of less than and greater than the EOF, respectively. Under the conditions used in this study, the EOF was about 2.5 min. In both the xylose–glycine and glucose–glycine systems the vast majority of compounds had longer migration times, suggesting that they possess a negative charge. This negative charge can be either because the MRP is anionic at pH 9.3, or because it forms an anionic complex with borate under these conditions. Borate is well known for its ability to form complexes with hydroxyl groups (Khun and Hoffstetter-Khun, 1993), and has been used as a complexing buffer in CE for the separation of sugars (Hoffstetter-Khun *et al.*, 1991; Morin *et al.*, 1993*a*,*b*; Honda, 1996) and oligosaccharides (Arentoft *et al.*, 1993). The tetraborate ion rapidly forms complexes with vicinal hydroxyl groups of sugars, resulting in negatively charged complexes which migrate towards the anode in CE. Thus, the migration time of an otherwise neutral sugar is changed. This complexation also results in an increased absorbance at 195 nm enabling the detection of sugars without derivatisation (Hoffstetter-Khun *et al.*, 1991).

If the charge on a compound is the result of borate complexation, then the concentration of borate ion in the buffer would be expected to affect the migration times of the compound. In order to examine this effect, the xylose–glycine 2-h reaction mixture was run in either borate or phosphate buffer pH 9.3, at buffer concentrations ranging from 50 to 100 mM. The migration times

of 2 resolved peaks (peak 1 which migrated near the EOF at 3.6 min, and peak 2 which had a long migration time of 8.3 min) and of the two melanoidin fractions are plotted against buffer concentration in Fig. 5. The migration time of peak 1, which possessed little negative character, was almost unaffected by increasing buffer strength in either borate or phosphate. There was, however, a dramatic increase in the migration time of peak 2 with increasing borate concentration, compared to very little change in migration time with increasing phosphate concentration. This is a clear indication that the migration time of this compound is affected by complexation with borate. For the two melanoidin fractions, increasing the buffer concentration had an effect on migration times in borate but almost no effect in phosphate buffer, indicating that borate complexation plays a role in the anionic migration character of melanoidins. Since Tomlinson *et al.* (1993, 1994) found that the majority of the MRPs in their model systems behaved as anions at low and high pHs, it is possible that some form of buffer complexation was contributing to this charge on the MRPs.

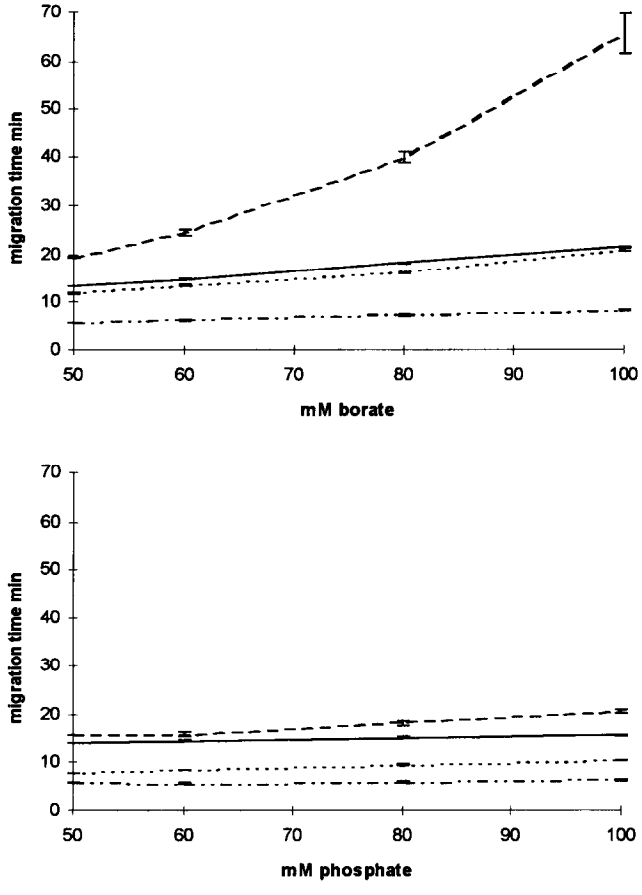


**Fig. 5.** Plot of CE migration time of various peaks obtained from the xylose–glycine model system, versus buffer concentration in either phosphate or borate buffer, run at 15 kV.—··— first large resolved peak (peak 1), migrating at 3.6 min in Figure 2 (f), ———— last large resolved peak (peak 2), migrating at 8.3 min in Figure 2 (f), ——— melanoidin fraction (molecular weight above 3000), ··· melanoidin fraction (molecular weight between 1000 and 3000). Standard errors shown.

## CONCLUSIONS

CE is a fast and efficient method for the separation and profiling of Maillard reaction products and many more components are resolved than by reverse phase HPLC. Ultrafiltration is a successful means of isolating the high molecular weight melanoidins which were shown to migrate as a single broad peak by CE. All other peaks detected by CE were shown to be due to smaller compounds with molecular weights of below 1000 daltons. The melanoidin and the majority of the lower molecular weight compounds migrated as anionic species at pH 9.3 in borate buffer. Part of this negative character was shown to be due to complexation with borate, suggesting the presence of hydroxyl groups on the MRPs. The majority of the colour in the systems was from the high molecular weight melanoidins, although a few of the low molecular weight components were also shown to absorb in the visible region.

## ACKNOWLEDGEMENTS

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