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Determination of gellan gum by capillary electrophoresis and CE–MS

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Abstract

Intact gellan gum (0.1% m/v) was detectable by capillary electrophoresis (CE) with UV detection. Characteristic tetrasaccharide fragments, prepared with a newly characterised gellan-degrading enzyme, provided a clearer signal that was detectable in complex food products containing other polysaccharides. Food products spiked with gellan gum could be analysed reproducibly with high accuracy and specificity by CE–ESI–MS, which is recommended as the technique of choice. Gellan gum declared as a fruit flavour drink ingredient could not be identified by CE–ESI–MS. When added to the product at the start of sample preparation, before enzyme treatment, the gum was readily detectable, demonstrating that the method was compatible with this sample type. Possible explanations for the negative results are that gellan gum was used as a trace component, with other texturing agents; that its declaration was precautionary only; or that the product contained a chemically modified form. Further work will establish whether modified gellan gums can be similarly analysed.

Keywords: Gellan gum; Capillary electrophoresis; UV detection

1. Introduction

Gellan gum, first reported in the late 1970s (Morris, 1989), is produced by pure culture fermentation of a carbohydrate from a bacterial strain originally designated as *Pseudomonas elodea* and now termed *Sphingomonas elodea*, and can be mass produced reproducibly (Winwood, Gibson, & Hoy, 1994). The use of gellan gum in foodstuffs is controlled in the UK by The Miscellaneous Food Additives Regulations 1995 (as amended). It is generally permitted for use in foods as a gelling, stabilising and suspending agent except where specific restrictions apply. Gellan gum used as a food additive must comply with the purity criteria set out in European Commission Directive 98/86/EC. In the UK it has been used in fruit pie products, coffee whitener and, recently, a fruit flavour drink product.

The objective of this work was to improve the specificity of gellan gum analysis. Established wet chemical tests for gellan gum (Baird & Smith, 1989; Graham, 1993) rely on a variety of precipitation techniques and colorimetric reactions. The most widely used reaction principle (Dische & Shettles, 1948) was originally applied to rhamnose, a component of gellan gum, but also of some other polysaccharides. One such method (Baird & Smith, 1989) was examined as a preliminary to the present work (results not shown). The Baird and Smith method was found to suffer from limited repeatability and reliability, under-recovery at lower gellan gum concentrations (a feature acknowledged by its originators), and a non-robust enzymic starch removal technique. It was known that agar and carrageenan interfered and needed to be separately identified by a supplementary method (Allen, Gardner, Wedlock, & Phillips, 1982), adding to the complexity and uncertainty of the procedure. Furthermore, the Baird and Smith method had not addressed a wide range of food products.

In consideration of all these issues, it became apparent that a radical new approach could contribute strongly to gellan gum analysis. The predominant

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molecular feature of gellan gum is a repeating tetrasaccharide structure containing a single carboxylic acid group (Jansson, Lindberg, & Sandforf, 1983; Morris, 1990):

-(1, 3) − β-D-glucose − (1, 4) − β-D-glucuronic acid

− (1, 4) − β-D-glucose − (1, 4) − α-L − rhamnose−

A polymer composed solely of such a structure should have a uniform mass-to-charge ratio regardless of its molecular size (neglecting chemical modification and stereochemical effects). This observation led to the choice of capillary electrophoresis (CE) as the instrumental analytical technique most likely to resolve gellan gum from complex mixtures such as food products.

CE (Ewing, Wallingford, & Olefirowicz, 1989; Gordon, Huang, Pentonay, & Zare, 1988; Li, 1992; Weinberger, 1993) has emerged over the past decade as a simple yet versatile, high resolution option challenging the established separation techniques. The detection limits of early commercial CE–UV absorbance detection systems were limited by the capillary diameter, but extended light path capillaries (Cole, Hiller, Chwojdak, & Sepaniak, 1996) have improved the situation. It is unusual to come across descriptions of food product formulations containing less than 0.01% m/v gellan gum. Non-specific CE–UV (i.e. without chemical derivatisation to enhance the measurement sensitivity at a specific wavelength for a particular compound) is now generally capable of detection at this level.

A gellan lyase enzyme was identified which cleaves gellan gum into its component tetrasaccharide repeat units (Hashimoto et al., 1997; Kennedy and Sutherland, 1994; Sutherland, 1999), although the enzyme was not yet commercially available. Whereas gellan gum molecules are heterogeneous in size, if the enzyme reaction was specific and reproducible the tetrasaccharide fragments produced by it would be identical; stereochemical effects on separative techniques would be eliminated, and mass spectrometric (MS) identification would be feasible.

MS has become an extremely powerful partner to LC, despite the fact that MS generally needs to operate at a much lower flow rate. CE offers MS-compatible flow rates, which will provide flexibility for the growth of CE–MS now that CE has itself become established technology. CE–MS provides high separation efficiency and accurate molecular mass information in a single analysis, giving increased confidence compared with the results obtained by CE–UV. Electrospray ionisation (ESI) has already gained wide recognition as the method of choice for interfacing CE with MS (Cai & Henion, 1995; Kirkby, Thorne, Gotzinger, & Karger, 1996). Advantages of ESI are its relative tolerance of non-volatile solutes, the wide range of interface models available (Cai & Henion, 1995), simplicity, ionisation efficiency, and spray stability. CE–MS has been used in the analysis of biological components, pharmaceutical drugs and drug metabolites (Cai & Henion, 1995), but very few publications to date have addressed real applications in food analysis.

2. Materials and methods

2.1. Materials and preparative methods

Food products were obtained from a local supermarket. Two and a half grams of each food product was initially diluted with 22.5 ml of water at 20°C, boiled for 5 min, then centrifuged for 1–10 min (as necessary) at 2300 ***g*** and 20°C. The supernatant was adjusted to pH 7.2±0.3 with dilute sodium hydroxide. A spike of gellan gum, if required, was then added (unless otherwise specified). Gellan gum was Kelcogel F (NutraSweet Kelco Company, Tadworth, UK; used only for early work with non-fragmented gum) or Gelrite Gellan Gum (Sigma-Aldrich Company Ltd, Poole, UK). Gellan lyase prepared at the University of Edinburgh as originally described (Kennedy & Sutherland, 1994) had an activity of approximately 5 μmol product/ml enzyme/3 h.

Gellan lyase digestion was typically performed by the rapid addition of 20 μl of the enzyme solution to a glass tube containing a mixture of 100 μl of digestion buffer (20 mmol/l tris-HCl, pH 7.2) with 100 μl of prepared food product or gellan gum standard solution. The total volume and the volume ratios of the reagents were kept constant within-run. The tubes were then transferred simultaneously to a water bath at 30±0.2°C for 3 h. At the end of this reaction period, the tubes were simultaneously boiled for 120±5 s. These heat-denatured solutions were diluted appropriately for CE and CE–MS, the dilution factor being kept constant within-run.

2.2. CE

A Hewlett Packard 3D CE (Waldbronn, Germany) was used with a Hewlett Packard Pentium II computer data station. CE was monitored using a diode array detector (DAD). A Hewlett Packard bubble factor 3 extended light path (BF3 ELP) fused silica capillary was used with total length 48.5 cm, effective length 40 cm, and internal diameter 50 μm (effective length is the distance from the detection window to the capillary inlet). Single or dual wavelength DAD detection was used routinely; spectral acquisition (190–390 nm) was used where appropriate.

CE runs were carried out in duplicate (each plot illustrated here is of a single run). The electric current output was routinely acquired as a function of time, to monitor data quality. CE–DAD electropherograms are

plotted with absorbance (milliabsorbance units, mAU) on the vertical axis and elution time (min) on the horizontal axis.

The following parameters were common to all CE–DAD experiments: capillary pre-conditioning with sodium hydroxide, 0.1 mol/l (1 min), water (1 min) and finally CE buffer (2 min); pressure-injection of sample and standards at 1150 mbar.s; positive potential at the capillary inlet with a 0.3 min rise time. Buffers were filtered (0.45 μm) before use.

2.3. CE Method A

Cassette temperature 35°C; CE voltage 15 kV. CE buffer: boric acid, 100 mmol/L, adjusted with sodium hydroxide to pH 8.0. DAD was conducted at 195 nm with a reference wavelength of 350 nm.

2.3.1. CE Method B

Cassette temperature 20°C; CE voltage 30 kV. CE–ESI–MS buffer was used as the electrolyte (see below). DAD was conducted at 228 nm and a reference wavelength of 350 nm.

2.4. CE–ESI–MS

The Hewlett Packard (HP) 3D CE unit was coupled to a HP MSD 1100 mass spectrometer with a Hewlett Packard Sprayer Kit (ESI interface). The CE capillary was a Hewlett Packard MS Fused Silica Capillary, total length 80 cm, internal diameter 50 μm. On-line CE–DAD (effective length 22.5 cm) was performed with ESI–MS. Replication and electric current monitoring were carried out as for CE–DAD. CE–ESI–MS data were acquired in cyclic scan mode (total ion current, TIC) or by selected ion monitoring (SIM), and are plotted with ion abundance on the vertical axis and elution time (min) on the horizontal axis.

CE–ESI–MS buffer was ammonium formate (analytical grade), 15 mmol/l, adjusted to pH 8.0 with ammonia solution (analytical grade, specific gravity 0.88). CE–ESI–MS sheath flow liquid was 2-propanol (HPLC grade):water 1:1 (v/v) with ammonia solution, 0.5%v/v. The mass calibrant for CE–ESI–MS was ES Tuning Mix (product G2421A obtained from H P, Waldbronn, Germany), a proprietary mixture of seven compounds ranging in molecular mass from 118 to 2722 dissolved in acetonitrile.

The optimised CE parameters for CE–ESI–MS were: cassette temperature 20°C; pressure injection of sample (500 mbar.s) followed by electrolyte buffer (250 mbar.s); 30 kV positive potential at the capillary inlet with a rise time of 0.3 min; DAD detection at 228 nm with a reference wavelength of 450 nm preceding MS on line. The ESI–MS parameters for CE–ESI–MS were: nebuliser pressure 10 psig; drying gas 6 l/min at 200°C; capillary voltage −3500 V; fragmentor voltage 100 V; ion mode, negative ESI; peak width 0.05 min; ion observation time in SIM mode, 280 ms. The observed capillary current was 0.4 μA.

3. Results and discussion

3.1. CE–DAD for gellan gum fragments produced by gellan lyase digestion

To identify suitable analytical parameters, the volume of gellan lyase solution incubated with gellan gum (0.1%m/v) was varied systematically. Gellan gum was recovered as a broad peak at 1.65 min, but an additional peak was observed at 1.54 min, which was named Fragment A (Fig. 1). Fragment A reached a plateau in peak height and did not increase proportionately in peak area as the volume of gellan lyase solution was increased beyond 5 μl. This lack of proportionality indicated that the new signal was not attributable to the gellan lyase protein itself, but rather to an enzyme digestion product. The absence of the Fragment A peak in the electropherogram obtained for gellan lyase without gellan gum supported this deduction (Fig. 1).

The gellan gum Fragment A peak occurs adjacent to the broad gellan gum peak, the latter therefore appearing as a shoulder. This arrangement would be expected if gellan gum molecules differing in molecular mass (and thus differing slightly in migration characteristics) were enzymically cleaved to release their common repeating tetrasaccharide unit. The tetrasaccharide is a sharply defined, low molecular mass boundary condition of the

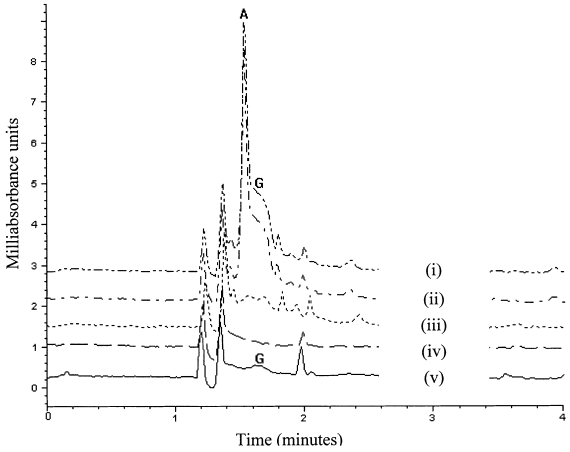

Fig. 1. CE–DAD electropherograms illustrating the formation of the characteristic Fragment A from gellan gum by digestion with gellan lyase. Duplicate electropherograms (i) and (ii) illustrate repeatability for gellan gum digested with 20 μl of gellan lyase. The remaining electropherograms illustrate control experiments: (iii) gellan lyase without gellan gum; (iv) blank; (v) gellan gum without gellan lyase. CE Method A was used (observation wavelength 195 nm). Gellan gum is marked 'G' and Fragment A is marked 'A'. A vertical offset has been used between electropherograms for clarity.

whole population of gellan molecules. This argument assumes that the fragment is not modified significantly by the cleavage process. It was anticipated that the action of gellan lyase might not be limited to the conversion of gellan gum to tetrasaccharide fragments of identical structure. In such conversion processes there might be side reactions, and precursor or successor products of the main product may be seen. It was therefore encouraging to observe no substantial digestion product peaks other than Fragment A. The formation of a well-defined product should assist in establishing a robust analytical method.

The absorbance spectrum of Fragment A peaked at 195 and 228 nm, but CE–DAD did not provide enough spectral information to characterise the chemistry of the fragment. Absorbance measurement at 228 nm was more selective for Fragment A among the considerable range of other materials present in the digestion mixture, and so this wavelength was used subsequently.

Gellan lyase, like other lyase enzymes, catalyses a highly specific β-elimination reaction (equivalent to chemical alkaline hydrolysis). Gellan gum is degraded by cleavage linked to the removal of the elements of water and the formation of an unsaturated bond between C4 and C5 of glucuronic acid residues, which recur in the polymer at intervals of four residues. The reducing ends of the fragments are left intact. Fragment A was therefore expected to be a dehydrated tetrasaccharide (Fig. 2) with a molecular mass of 646, based on addition of the integer atomic mass values.

3.2. CE–DAD analysis of food products

Five food products were selected which contained no declared gellan gum: ready to eat strawberry flavour jelly; concentrated orange squash; tomato ketchup; strawberry jam; and coleslaw. The products were also selected to include potential carbohydrate interferents in the study, such as sugar and/or texturing agents including carob gum, carrageenan, sodium alginate, pectin, guar gum and xanthan gum. The CE method underwent further development at this stage as the borate electrolyte was replaced with a volatile ammonium formate electrolyte suitable for CE–ESI–MS (see below). The gellan gum spike was detected in all five food products as a characteristic peak with a mean migration time of 2.27 min (coefficient of variation 3.3% over six matrix materials including the aqueous control; illustrative data are given in Fig. 3). This peak was assumed to contain Fragment A, as identified earlier under different CE conditions.

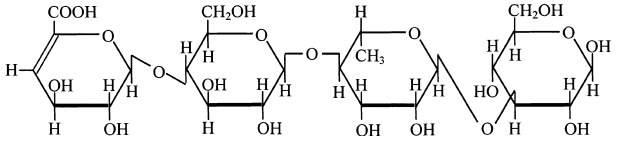

Fig. 2. Expected chemical structure of gellan gum Fragment A.

The interpretation of unknown samples by the CE method of Fig. 3 would require considerable skill, because of background peaks from the food products as well as from the gellan lyase preparation itself (although the latter problem will be addressed by enzyme purification techniques). In principle, it would be possible to analyse each new sample with and without gellan lyase treatment, and to align the common peaks, or peaks from added standards, so that Fragment A could be identified and estimated with a high degree of confidence from the difference spectrum. Such an alignment procedure could be automated through pattern recognition software.

3.3. CE–MS method development to improve the confidence of gellan gum determination

Hyphenation of CE with MS was established using the existing CE instrument coupled through an ESI interface module to a bench top quadrupole mass spectrometer. The chemical identity of Fragment A (molecular mass 646, Fig. 2) was supported by CE–ESI–MS with selected ion monitoring (SIM), which detected a signal of m/z 645, equal to the expected carboxylate monoanion (Fig. 4). The migration time for this ion was greater than that obtained by on-line CE–DAD because of the difference in distance travelled by the ions along the capillary for the two detection methods.

Gellan lyase digests of gellan gum prepared independently were also analysed (Fig. 5). Two substantial peaks, similar to one another in their migration times,

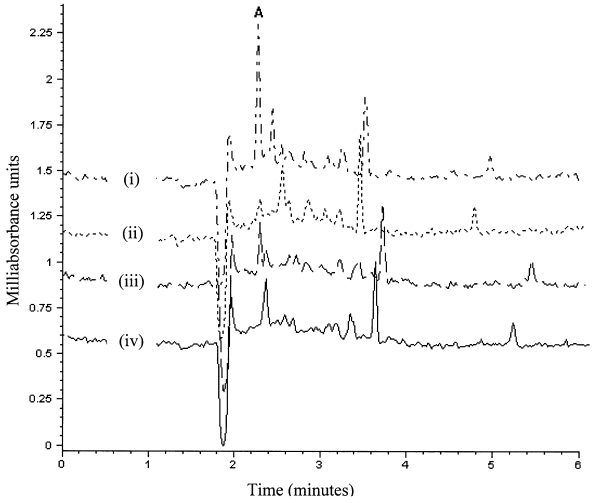

Fig. 3. Illustrative CE–DAD electropherogram for a jelly product with 0.2% m/v gellan gum added, following gellan lyase treatment. CE Method B was used. Fragment A is marked 'A'. (i) Gellan gum with active gellan lyase; (ii) no gellan gum, active gellan lyase; (iii) gellan gum with denatured gellan lyase (heat-inactivated at 100°C for 2 min); (iv) no gellan gum, denatured gellan lyase. Vertical offsets between electropherograms have been applied for clarity.

were observed by CE–DAD and CE–ESI–MS in scan mode. An ion abundance versus m/z plot for this region of the electropherogram contained two principal ions of m/z 645 and 483. The principal peak in the electropherogram was associated with the ion of m/z 645, and the second peak was similarly associated with the ion of m/z 483; no other significant components were observed. The signal-to-noise ratio was calculated as a measure of the detection limit for on-line CE–DAD and CE–ESI–MS (SIM at m/z 645) for the experiment shown in Fig. 5. On this basis, the detection limit of CE–ESI–MS for gellan gum was 40 times better than that of CE–DAD, the former providing an analytical detection limit of 81 μg kg^{-1} for gellan gum based on a signal to noise ratio of 3:1.

Comparison of Figs. 4 and 5a illustrates that the migration time of Fragment A (m/z 645) has altered between experiments. This is allowable at the present stage of development of CE technology provided that, as demonstrated below (Fig. 6), consistency of migration times is obtained within an analytical set comprising samples and appropriate quality assurance materials. A stringent daily rinsing procedure helps to control variations arising from capillary surface interactions. Development of a high performance technique is in progress, including a pre-conditioning protocol for new capillaries, daily capillary rinse procedure, capillary outlet adaptation and ion spray stabilisation.

3.4. CE–MS analysis of food products

Spiked and non-spiked food products formerly analysed by CE (Fig. 3) were now reanalysed by CE–ESI–MS (Fig. 6). In all cases, Fragment A could be clearly distinguished in SIM mode as a sharp peak of m/z 645. The mean migration time for Fragment A of 5.84 min was highly repeatable within each food matrix (coefficient of variation 0.5%, pooled for five sample types — Fig. 6). Between food products, the mean migration time was also reproducible (coefficient of variation 3.6% over five sample types — Fig. 6). CE–ESI–MS was therefore suitable for the determination of gellan gum in a range of diverse food products. In the case of an uncharacterised food matrix, the presence of gellan gum could be confirmed, even if the migration time was altered, by comparison of CE–ESI–MS runs performed with and without gellan lyase treatment of the sample, as discussed above for CE–DAD analysis.

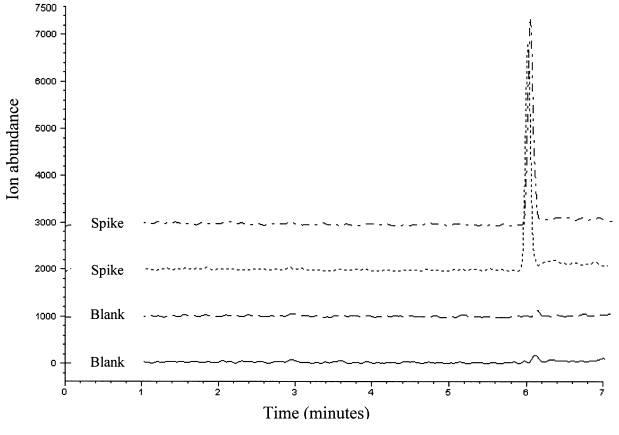

Fig. 4. CE–ESI–MS: SIM (m/z 645) of Fragment A. Duplicate runs are shown of samples with and without aqueous gellan gum, treated with gellan lyase at LGC. The electropherograms are offset against the vertical axis for clarity.

The identification of gellan gum by CE–ESI–MS having been demonstrated, quantification was investigated. The dose–response relationship was similar for the spiked food product and the corresponding aqueous standards (Table 1). A calibration curve will probably be required to maximise the accuracy of the determination, because there appeared to be some association between the concentration of spike and the recovery over the concentration range of interest (0.01–0.2% m/v in food).

3.5. Evaluation of a food product declaring gellan gum as an ingredient

Only one retail food product — a fruit flavour drink (FFD) — was known to be labelled as containing gellan gum. FFD was also labelled as containing modified starch, guar gum and xanthan gum. Fragment A could not be detected in FFD treated with gellan lyase and analysed by CE–ESI–MS and CE–DAD. Gellan lyase-treated aqueous gellan gum was then added to the gellan lyase-treated FFD, and was successfully recovered from the food matrix as the CE–ESI–MS Fragment A peak of m/z 645, migration time 5.45 min. This

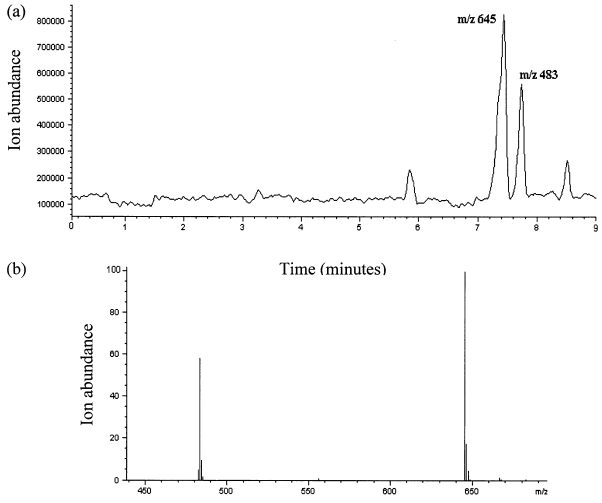

Fig. 5. Analysis of gellan lyase-treated gellan gum prepared independently (at Edinburgh University). (a) CE–ESI–MS total ion current electropherogram. (b) Ion abundance versus mass-to-charge ratio summed over the two principal peaks of (a) and excluding signals from outside this migration time window.

demonstrated that the FFD matrix does not suppress the Fragment A signal if the latter substance is added directly at a high enough concentration.

Until this point, the target detection range for gellan gum in food products was considered to be 0.01–0.3% m/v, based on the concentration range in which the gum would have useful rheological modification properties. However, it is possible that gellan gum would be blended so as to make up only a small percentage of the total polysaccharide content in a complex product such as FFD. The concentration of gellan gum in the complete product could therefore be slightly less than 0.01% m/v. To maximise the concentration of any gellan gum present, non-spiked gellan lyase-treated FFD was freeze dried, then reconstituted with a smaller volume of water, giving a concentration factor of 2.8, for re-ana-

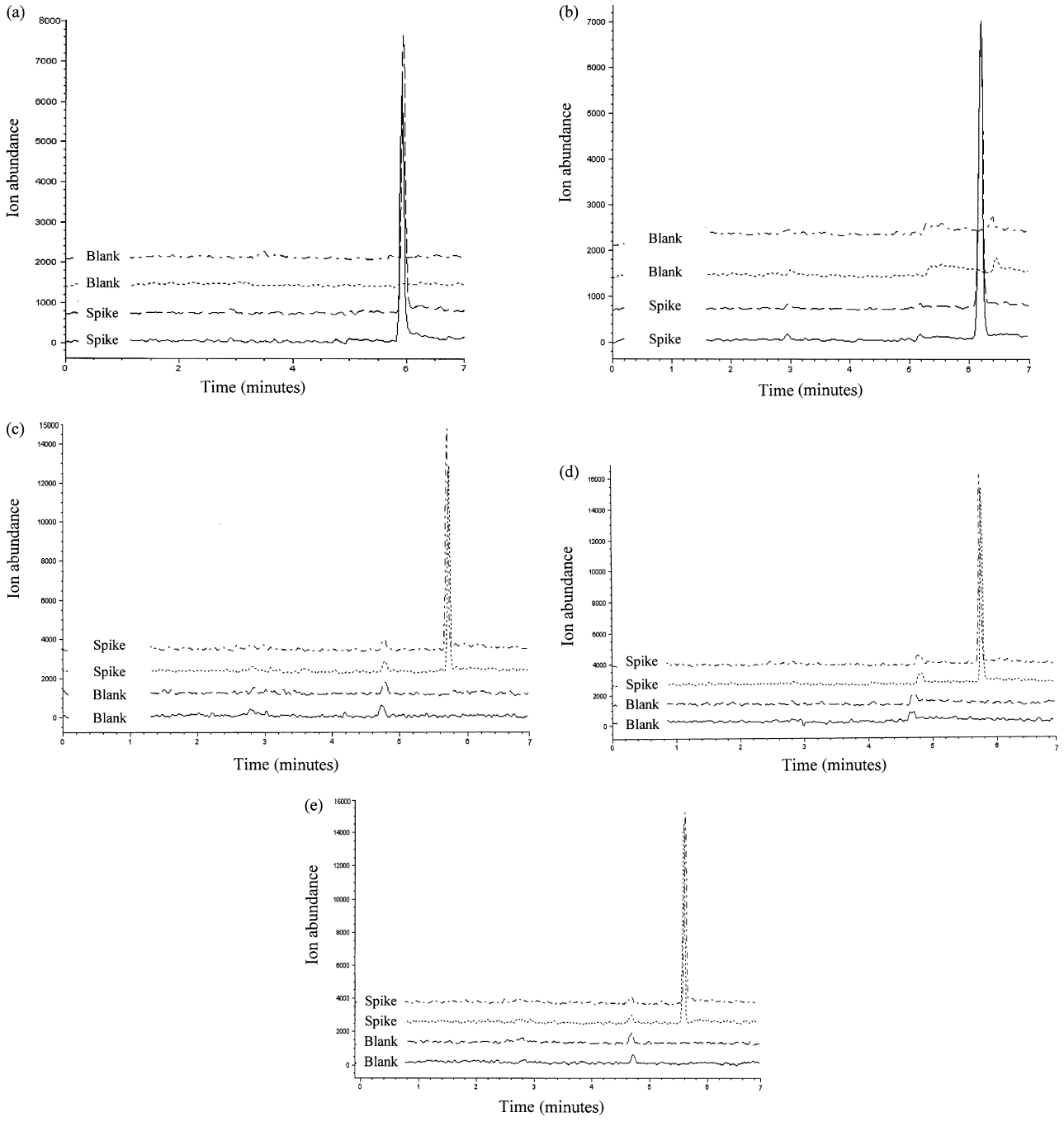

Fig. 6. CE–ESI–MS SIM mode (m/z 645) abundance versus migration time profiles for five gellan lyase-digested food products spiked with 0.2% m/v gellan gum. This figure shows new analyses of samples digested alongside the one for which CE–DAD analysis was illustrated in Fig. 3. Each diagram shows duplicate determinations of spiked and non-spiked samples; the prominent peak in each case corresponds to gellan gum. A vertical offset has been applied between electropherograms for clarity. (a) jelly; (b) orange drink; (c) tomato ketchup; (d) jam; (e) coleslaw.

Table 1
CE–ESI–MS dose-response data for Fragment A produced by gellan lyase treatment of gellan gum-spiked jelly and of aqueous gellan gum[a]

Sample type	Gellan gum (% m/v)	Peak area			Peak height		
		Mean	Recovery	CV	Mean	Recovery	CV
Aqueous gellan gum	0	0	na	0	0	na	0
	0.01	2168	80	2.3	706	76	2.2
	0.05	11,558	85	1.6	3860	83	0.4
	0.2	54,442	100	3.6	18,583	100	3.6
Jelly spiked with gellan gum	0	0	na	0	0	na	0
	0.01	2416	89	18.6	627	67	15.3
	0.05	11,565	85	0.8	3894	92	0.3
	0.2	49,547	91	1.0	16,955	91	0.4

[a] Gellan gum was spiked in jelly at the tabulated concentrations, and aqueous gellan gum was analysed at the same concentrations. The recoveries are percentages calculated relative to the highest concentration of aqueous gellan gum from duplicate runs. CV, coefficient of variation; na, not applicable.

Table 2
Recovery from enzyme-digestion CE–ESI–MS of gellan gum added to the fruit flavour drink product FFD at the start of sample preparation[a]

Gellan gum (% m/v)	Mean peak area in FFD	CV of peak area in FFD	Recovery in FFD (%)
0	0	0	na
0.01	2700	6.6	73
0.02	5500	8.3	46
0.05	12,300	2.9	43
0.1	17,800	0.9	24
0.15	29,100	0.9	29
0.2	37,300	3.0	29

[a] The tabulated concentration series were prepared in duplicate in FFD, and in duplicate as standards in water. Gellan gum was added before the initial sample dilution step (see Section 2). CV, coefficient of variation; na, not applicable. The recovery for each point is calculated directly from the standard of the same concentration.

lysis by CE–ESI–MS in SIM mode (m/z 645). A broad peak of migration time 6.7 min was observed from the freeze-dried FFD, a substantially greater migration time than from the same material before freeze drying. It is common for a concentrated sample matrix to increase the CE migration time of a target analyte. However, it is more likely that the peak at 6.7 min corresponded to a peak of similar shape and migration time observed before freeze drying, and that this peak did not contain gellan gum. It was not possible to conclude from these experiments whether FFD contained gellan gum.

It was proposed that FFD could contain endogenous enzyme inhibitors, preventing the formation of Fragment A from gellan gum. This possibility was tested by digestion of gellan gum-spiked FFD followed by CE–ESI–MS; the experiment confirmed at three concentrations (0.0125–0.25% m/v gellan gum in matrix) that the FFD matrix did not prevent Fragment A production. Finally, a further control experiment confirmed that gellan gum could be extracted from FFD when added at the beginning of the sample preparation procedure (Table 2). The recovery was incomplete, but would certainly be high enough for the detection of gellan gum in the targeted concentration range.

4. Conclusion

An enzymic lyase digestion fragment of gellan gum can be observed by CE with optical detection in a representative range of spiked food products containing sugar and texturing agents. These spiked food products can be analysed reproducibly and unambiguously by CE–ESI–MS with selected ion monitoring. CE–MS offers the strongest prospects for rigorous, highly selective determination of gellan gum in food products, although CE with optical detection may be more available to laboratories in the short term.

Gellan gum was declared as an ingredient of a widely available fruit flavour drink, but was not detectable in this product by CE–MS. Control experiments showed that the sample preparation and enzyme digestion procedures recovered gellan gum effectively from this product. Possible explanations are that the additive is only present at a trace level, perhaps as a minor component of a blend of texturing agents; that the additive was declared only as a precautionary measure, for instance if the recipe was subject to modification; or that a chemically distinct analogue of gellan gum was used in the product. Further work will focus on related compounds

and, if necessary, on improved detection limits. CE and MS technology offer great scope for modernising the whole area of polysaccharide analysis, in some cases with the use of other specific degradative enzymes such as the xanthanases (Sutherland, 1994).

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References

Allen, J. C., Gardner, R., Wedlock, D. J., & Phillips, G. O. (1982). The use of 2-thiobarbituric acid in the assay of κ-carrageenan in polysaccharide mixtures. *Progress in Food Nutrition and Science, 6*, 387–392.

Baird, J. K., & Smith, W. W. (1989). An analytical procedure for gellan gum in food gels. *Food Hydrocolloids, 5*, 407–411.

Cai, J., & Henion, J. (1995). CE-MS review. *Journal of Chromatography A, 703*, 667–692.

Cole, O. R., Hiller, L. D., Chwojdak, A. C., & Sepaniak, M. J. (1996). Evaluation of extended-light path capillaries for use in CE with LIF detection. *Journal of Chromatography A, 736*, 239–245.

Dische, Z., & Shettles, L. B. (1948). A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination. *Journal of Biological Chemistry, 175*, 595–603.

Ewing, A. G., Wallingford, R. A., & Olefirowicz, T. M. (1989). Capillary electrophoresis. *Analytical Chemistry, 61*, 292A–303A.

Gordon, M. J., Huang, X., Pentoney, S. L., & Zare, R. N. (1988). Capillary electrophoresis. *Science, 242*, 224–228.

Graham, H. D. (1993). Mg^{2+} selectively isolates gellan gum from dairy products. *Journal of Food Science, 58*, 539–543, 566.

Hashimoto, W., Maesaka, K., Sato, N., Kimura, S., Yamamoto, K., Kumagai, H., & Murata, K. (1997). Microbial system for polysaccharide depolymerization: enzymatic route for gellan depolymerization by *Bacillus* sp. GL1. *Archives of Biochemistry and Biophysics, 339*, 17–23.

Jansson, P.-E., Lindberg, B., & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydrate Research, 124*, 135–139.

Kennedy, L., & Sutherland, I. W. (1994). Gellan lyases — novel polysaccharide lyases. *Microbiology, 140*, 3007–3013.

Kirkby, D. P., Thorne, J. M., Gotzinger, W. K., & Karger, B. L. (1996). A CE/ESI-MS interface for stable, low flow operation. *Analytical Chemistry, 68*, 4451–4457.

Li, S. F. (1992). *Capillary electrophoresis principles and applications*. Amsterdam: Elsevier Science.

Morris, V. (1989). New ways of structuring food. *Food Flavourings, Ingredients, Packaging and Processing, 11*, 47–49,51.

Morris, V. J. (1990). Biotechnically produced carbohydrates with functional properties for use in food systems. *Food Biotechnology, 4*, 45–57.

Sutherland, I. W. (1994). Enzymic methods for the structural analysis of xanthans. In J. N. BeMiller, D. J. Manners, & R. J. Sturgeon, *Methods in carbohydrate chemistry*, Volume 10 (Chapter 32, pp. 199–206). John Wiley. ISBN 0471529400.

Sutherland, I. W. (1999). Polysaccharases for microbial exopolysaccharides. *Carbohydrate Polymers*, (in press).

Weinberger, R. (1993). *Practical capillary electrophoresis*. New York: Academic Press Inc.

Winwood, R. J., Gibson, W., & Loy, S. M. (1994). Gellan gum and its novel texture. In G. G. Birch, & G. Campbell-Platt, *Synergy* (pp. 105–117). Andover: Intercept Ltd.