A Sensitive and Selective Method for the Analysis of Complex Mixtures of Sugars and Linear Oligosaccharides

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2-Aminoacridone has been used to derivatise a variety of sugars and polysaccharides *via* the formation and reduction of a Schiff base. Separation of these carbohydrate derivatives was achieved with high selectivity and sensitivity using micellar electrokinetic capillary chromatography (MECC) followed by laser induced fluorescence detection.

Carbohydrates play an important role in nature and can have diverse functions. Besides forming part of the intricate architecture of cells, they can also be intimately involved in the biological function of glycoproteins.[1] Thus much research effort has been dedicated towards the development of analytical techniques for the separation and detection of the components of complex carbohydrate mixtures. Unfortunately the absence of chromophores in most carbohydrates renders them transparent to direct detection by UV absorbance. Hence anion-exchange high performance liquid chromatography (HPLC) at a highly alkaline pH followed by pulsed amperometric detection is presently the most common methodology for the analysis of sugar residues and complex oligosaccharides.[2]

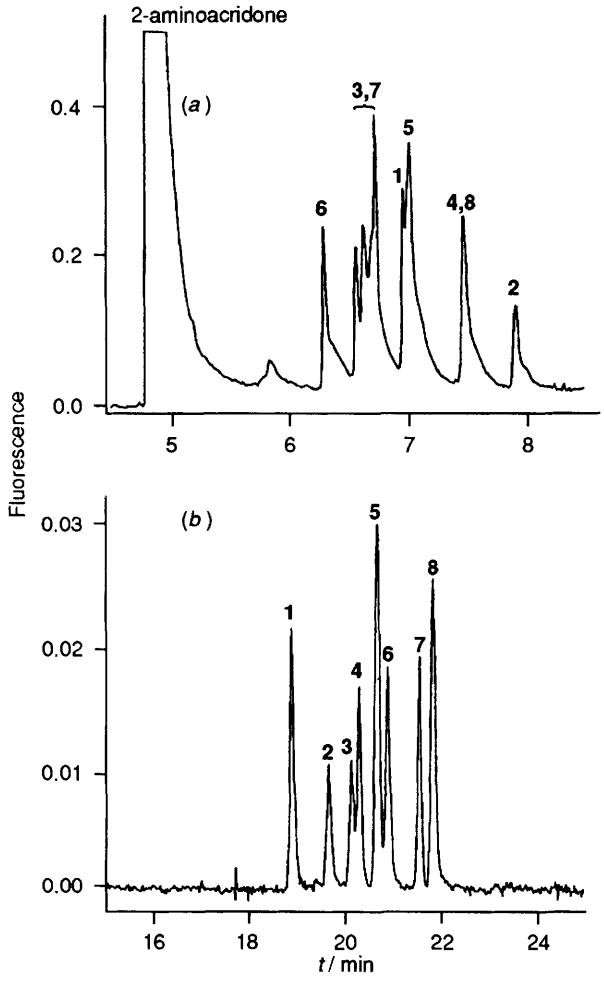

Fig. 1 Electropherograms of aminoacridone-derivatised sugars using (*a*) free zone capillary electrophoresis and (*b*) MECC with 80 mmol dm^{-3} taurodeoxycholate. *Conditions*: Fused-silica capillary, 570 mm × 50 μm, 300 mmol dm^{-3} borate buffer, pH 8.8; 20 °C; voltage, 25 kV; sample injection, 1 s of a solution, *ca.* 1 mmol dm^{-3} in water. Derivatised sugars (identified by co-electrophoresis with authentic standards): **1**, *N*-acetylglucosamine; **2**, galactose; **3**, mannose; **4**, fucose; **5**, glucose; **6**, *N*-acetylgalactosamine; **7**, lyxose; **8**, ribose. Excitation at 488 nm, emission at 520 nm.

A number of methods for the analysis of carbohydrate material have also been developed involving derivatisation with radiolabelled, UV-absorbing or fluorescent compounds. Such derivatisations are usually carried out using molecules that can easily undergo a Schiff's reaction with the aldehyde form of the sugar to give intermediates which are directly reduced to stable products, these include 2-aminopyridine and 6-aminoquinoline.[3,4]

Derivatised carbohydrates have been traditionally analysed by HPLC. The more recent development of capillary zone electrophoresis (CZE) has opened new possibilities for the selective analysis of complex mixtures of these carbohydrate derivatives. Open-tubular or free-zone capillary electrophoresis has been to date the preferred mode of CZE for the resolution of these molecules. Separations are normally carried out in the pH range of 8 to 12 using borate buffers. These play a dual function; buffering the medium and forming negatively charged complexes with carbohydrates. Both UV and laser induced fluorescence (using the 325 nm line of a helium–cadmium laser as the excitation wavelength) have been used to detect carbohydrates derivatised with aminopyridine compounds or aminonaphthalene sulfonic acid related reagents.[5,6]

Recently, Jackson[7] described the use of the fluorophore 2-aminoacridone for the analysis of reducing carbohydrates by polyacrylamide slab gel electrophoresis. We now describe the use of micellar electrokinetic capillary chromatography (MECC) for the separation of a number of sugars and polysaccharides derivatised by this reagent. Analytes were conveniently detected by an argon laser, operating at a wavelength of 488 nm, as the excitation source, measuring the fluorescence at 520 nm.

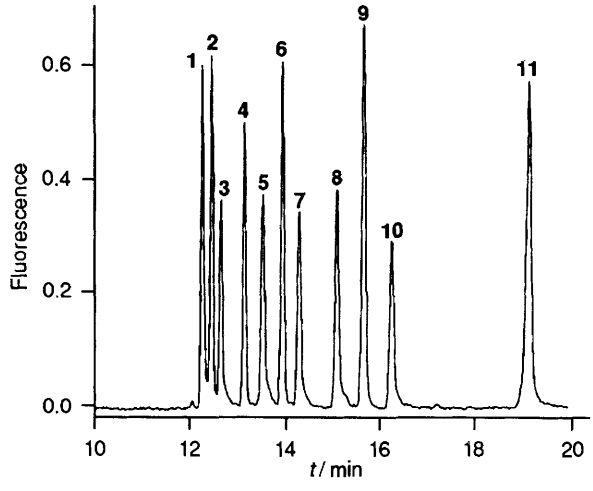

Fig. 2 Electropherogram of a mixture of the aminoacridone derivatives of glucose, four cello-oligosaccharides and seven malto-oligosaccharides. MECC conditions are the same as in Fig. 1(*b*). The difference in migration time for glucose in Fig. 1(*b*) and this Fig. is primarily due to different capillaries being used in the two experiments. Derivatised compounds: **1**, maltoheptose; **2**, cellopentose; **3**, maltohexose; **4**, cellotetrose; **5**, maltopentose; **6**, cellotriose; **7**, maltotetrose; **8**, maltotriose; **9**, cellobiose; **10**, maltose; **11**, glucose. Excitation at 488 nm, emission at 520 nm.

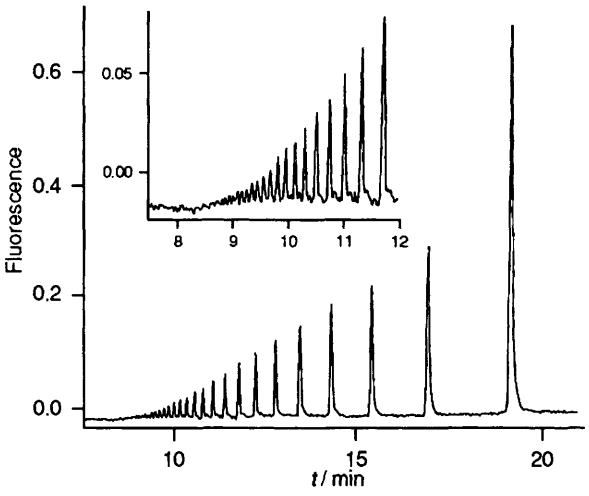

Fig. 3 Separation of the derivatised components of a 'dextran ladder'. MECC conditions as in Fig. 2. Inset shows an expansion of the timescale from 7.5 to 12 min. Excitation at 488 nm, emission at 520 nm.

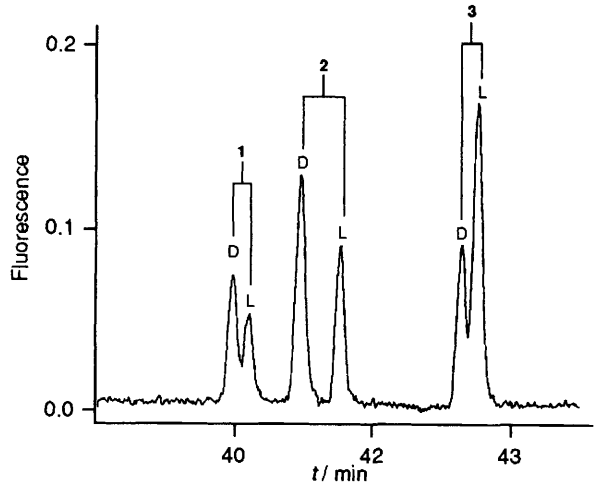

Fig. 4 Resolution of monosaccharide enantiomers derivatised with aminoacridone. Borate buffer (500 mmol dm^{-3}) pH 8.8 contained 80 mmol dm^{-3} taurodeoxycholate and 15 mmol dm^{-3} β-cyclodextrin. Capillary length, 970 mm; *T*, 30 °C; voltage, 30 kV. Derivatised sugars: **1**, D,L-galactose; **2**, D,L-fucose; **3**, D,L-ribose. Unequal concentrations of each antipode were injected to facilitate identification. Excitation at 488 nm, emission at 520 nm.

The separation of eight aminoacridone derivatised sugars (*N*-acetylglucosamine, galactose, mannose, fucose, glucose, *N*-acetylgalactosamine, ribose and lyxose) using open tubular CE is shown in Fig. 1(*a*). Resolution is unsatisfactory, peak-shape is poor, and the migration of excess reagent occurs close and prior to that of the analytes of interest. The introduction of a surfactant such as the sodium salt of taurodeoxycholate in the buffer solution changes the order of migration of these analytes and enhances their resolution to a great extent [Fig. 1(*b*)]. Moreover, the migration time of the aminoacridone (not shown) is about 10 min after that of the last derivatised sugar, that is lyxose. Similar results are obtained on the addition of sodium dodecyl sulfate (SDS) to the buffer solution.

2-Aminoacridone will be largely neutral below a pH of about 9, the expected pK_a value for the dissociation of the heterocyclic NH group in this molecule. Thus, uncharged fluorophore can partition into the taurodeoxycholate micelle more effectively than the more hydrophilic, derivatised sugars. Evidence for this micelle incorporation was obtained by the observation that the fluorescence emission from this compound at 520 nm increased by 45% on addition of surfactant to the buffer solution.[8]

The mechanism of migration of the derivatised sugars is complex and may involve partitioning equilibria of the neutral species across the micellar surface and electrophoretic movement of these molecules complexed with borate. Electroosmotic flow will also effect both the migration of the micelle and that of the borate complexes.

Fig. 2 shows the separation of a mixture of eleven analytes made up of the aminoacridone derivatives of glucose, six malto-oligosaccharides and four cello-oligosaccharides. The linkage between the glucose units in the maltose related molecules is α-1–4 whereas that in the cello-oligosaccharides is β-1–4. Despite the closely related structures of the linear oligosaccharides in Fig. 2, resolution of their aminoacridone derivatives by MECC is excellent. Although dependence of migration time on conformation cannot be excluded, the order of migration appears to be directly related to size: the higher oligomers migrate first and glucose (the smallest unit) last, a situation similar to that observed in size exclusion chromatography.

The separation of the derivatised components of a 'dextran ladder' is illustrated in Fig. 3. This mixture was again found to extend from the smallest possible unit, that is one glucose molecule, to about twenty seven glucose units. The baseline resolution obtained for all the components in this mixture and the lack of interference from excess 2-aminoacridone itself are remarkable.

To further demonstrate the utility of aminoacridone derivatisation and MECC analysis we used this methodology to separate the enantiomeric forms of some sugars. Results are shown in Fig. 4. Addition of β-cyclodextrin to the separation buffer is necessary for chiral discrimination. Moreover, replacement of taurodeoxycholate by a non-chiral surfactant such as SDS does not result in the resolution of the pairs of enantiomers shown. This result is in agreement with data we reported[9] recently showing that interaction between β-cyclodextrin and taurodeoxycholate can give rise to enhanced chiral separations in MECC.

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