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Ethanol determination using pressure mobilization and free solution capillary electrophoresis by photo-oxidation assisted ultraviolet detection☆

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ABSTRACT

Free solution capillary electrophoresis (CE) can separate and quantify carbohydrates using a simple direct UV detection based on a photo-oxidation reaction taking place in the detection window without any labeling. Ethanol interferes with this photo-oxidation reaction. We thus present the first detection and quantification of ethanol using either a simple pressure mobilization set-up or CE. Ethanol can be detected down to 34.9 mg L^{-1} and quantified in the range 117–1850 mg L^{-1} through the interference with photo-oxidization of 2 g L^{-1} sucrose. CE can thus separate and quantify both carbohydrates and ethanol, for example to monitor a lignocellulosic fermentation process. The method is not limited to ethanol and applies to alkyl amines and other alcohols and likely to most molecules possessing the ability to react with free radicals assuming they can be sufficiently separated from each other.

1. Introduction

The determination of ethanol is essential for the analysis of ethanol fermentations and related alcoholic beverages. Bioethanol fermentation of lignocellulosic material is an important process that will hopefully significantly reduce the global demand on fossil fuels. Available methods for the detection and quantification of ethanol in complex matrices are few. Typical methods for the determination of ethanol include Gas Chromatography with Flame Ionization Detection (GC–FID) [1], High Performance Liquid Chromatography (HPLC) with Refractive Index detection (RI) on a cation exchange resin [2] or High Performance Anion Exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD) [3]. The detection of underivatised ethanol is challenging due to its lack of UV absorption or fluorescence emission. In ethanol fermentation, an analytical method is more advantageous if it can determine both carbohydrates and ethanol. GC methods require derivatization of carbohydrates to make them volatile like ethanol [4], while HPLC [5] and HPAEC have high running costs and, in the case of HPLC, may suffer from poor robustness and recovery [5]. Additionally, no single separation technique can determine simultaneously ethanol and a complex mixture of carbohydrates in a complex matrix such as that of a lignocellulosic fermentation.

Some modes of Capillary Electrophoresis (CE) have previously been used in the detection of ethanol. Ethanol and other solvents have been previously quantified by Micellar ElectroKinetic capillary Chromatography (MEKC) with indirect detection [7] however it requires the use of sodium dodecyl sulfate (SDS) surfactant that may interact with proteins and lipids present in complex samples, such as lignocellulosic fermentations. CE with PAD [8] or indirect UV detection [9] was able to detect ethanol however no quantification was carried out. CE with direct UV detection at high pH is a simple and robust method developed for carbohydrate analysis [10]. The separation has been applied to wide variety of complex matrices including forensic, food, beverage and pharmaceutical samples [6], fruit juices and cognac [10], and complex acid treated plant fiber samples [5,11]. Ethanol was however never determined by this method limiting its application for monitoring ethanol fermentations. Direct UV detection of carbohydrates in CE at high pH was originally suggested to be due to enediolate

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formation [6,10] but later shown to be due to be a photo-oxidation reaction [5,6]. This photo-oxidation reaction takes place directly in the detection window [12,13]. Hydroxyl and/or superoxide related radicals may be produced following minimal but sufficient decomposition of water at the low wavelength UV irradiation in the detection window [14], then react with the carbohydrates. Alternatively the carbohydrates may be directly photo-decomposed by the irradiation [15]. In both cases the radicals obtained from the carbohydrates decompose through a pathway containing UV-absorbing (250–270 nm) intermediates [13,16] to carboxylate decomposition products [13]. Multiple passes through the detection window in one experiment (reversing the electric field after each pass) revealed that the electrophoretic mobility of the carbohydrates is constant but the peak intensity increases for 6 passes before decreasing [5]. The UV absorption at 250–270 nm is observed with a CE Diode Array Detector (DAD) but not with a classical grate spectrophotometer [12]. Ethanol might also encounter some photo-oxidation under these conditions [17], but it does not lead to any UV absorption. Ethanol undergoes hydrogen abstraction in the presence of some free radicals e.g. in the presence of peroxides [18] or in radical polymerization [19,20]. We hypothesized that ethanol would interfere with the photo-oxidation reaction and hence the detection of the carbohydrate and this interference would lead to a change in direct UV detection in carbohydrates proportionally to the amount of ethanol present. The aim of this study was to investigate a detection method for ethanol with free solution CE equipment compatible with both pressure mobilization and free solution CE through its interference with the photo-oxidation reaction, to investigate how ethanol could interfere with the detection of carbohydrates and to apply the detection method to fermentation samples and alcoholic beverages. The long term goal is to develop a separation method that can determine both ethanol and carbohydrates in a complex sample such as the fermented lignocellulosic plant fiber.

2. Materials and methods

2.1. Materials

Water was of MilliQ quality (Millipore, USA). Sodium hydroxide pellets (NaOH) ≥98%, absolute ethanol ≥99.5% and magnesium chloride hexahydrate ≥99% were obtained from Sigma–Aldrich (Australia). Xylitol ≥99%, sucrose ≥99% and ammonium sulfate 99% were obtained from Alfa Aesar (USA). Fused-silica capillaries (50 μm i.d., 360 μm o.d.) were obtained from Polymicro (USA). Triethylamine ≥99.5% and *tert*-butanol ≥99% was obtained from BDH (UK). Deuterium oxide (D, 99.9%) and fully ^{13}C-labeled glucose ≥99% were sourced from Cambridge Isotope Laboratories (USA). Yeast extract was obtained from Oxoid (Australia). Monopotassium phosphate ≥99% was obtained from Univar (Australia). Vodka (declared alcohol content 37%) was produced commercially in Australia and purchased locally. The fermentation sample, after dilution, was comprised of 500 mg L^{-1} of each glucose, fructose and yeast extract and of 0.50 mg L^{-1} of each $MgCl_2$, $(NH_4)_2SO_4$ and KH_2PO_4.

2.2. Free solution capillary electrophoresis (CE) and pressure mobilization

The instruments were a MDQ P/ACE (Beckman) and a 7100 CE (Agilent) with DADs monitoring at 200 nm and 266 nm with a 10 nm bandwidth. Samples were injected by applying 14 mbar of pressure for 8 s (≈10 nL) followed by mobile phase or background electrolyte (BGE) injected in the same manner. At the end of a series of injection, the capillary was flushed 1 min with NaOH 1 M, 10 min with water and 10 min with air. Integration was performed using Karat 32 (Beckman) or Chemstation (Agilent) software.

2.2.1. Pressure mobilization

The capillary length was 90 cm with an effective length of 10 cm on the MDQ P/ACE (Beckman) and of 8.5 cm on the 7100 CE (Agilent) instruments. The mobile phase was comprised of 130 mM NaOH unless otherwise specified. Sucrose and xylitol do not reduce in the presence of 130 mM NaOH in water and therefore their solutions were prepared in such medium. The capillary was pre-treated prior to use and between each run by flushing with the mobile phase for 5 min. Pressure mobilization was at 50 mbar unless otherwise specified. For NMR spectroscopy, 1 g L^{-1} of ^{13}C labeled glucose and 2 g L^{-1} of ethanol in 130 mM NaOH in D_2O was pressure injected continuously at 50 mbar into a 35 cm capillary (26.5 cm effective length) on the 7100 CE instrument with the lamp on; 130 μL was collected, and made up to 580 μL with 130 mM NaOH in D_2O.

2.2.2. Free solution capillary electrophoresis (CE)

The capillary length was 90 cm with an 81.5 cm effective length on the 7100 CE (Agilent). The BGE consisted of 130 mM NaOH with 2 g L^{-1} of sucrose in the capillary and 130 mM NaOH only, in the inlet and outlet vials. The capillary was pre-treated prior to use by flushing with 1 M NaOH for 20 min followed by water for 5 min then the BGE for 10 min. The BGE containing sucrose was then flushed between injections for 10 min. The electric field was applied for 12 min at 24 kV followed by pressure mobilization at 50 mbar.

2.3. NMR

^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker DRX300 spectrometer (Bruker, Alexandria, NSW, Australia) operating at 300 MHz for ^{1}H, equipped with a 5 mm ^{1}H–^{13}C dual probe. ^{1}H NMR spectra were recorded with a 5.3 μs 30° pulse, a 5 s repetition delay and 800–20,480 scans. ^{13}C NMR spectra were recorded with a 7 μs 90° pulse, a 3 s repetition delay and 20,358–184,320 scans. ^{1}H and ^{13}C chemical shift scales were externally calibrated with the resonance of the methyl signal of ethanol in D_2O at 1.17 and 17.47 ppm, respectively [21].

3. Results and discussion

3.1. Photo-oxidation assisted detection of ethanol

Pressure mobilization of sucrose dissolved in 130 mM NaOH led to a Gaussian peak, which intensity decreased when ethanol was added to the sucrose (Fig. 1A). Sucrose peak area, height and shape are increasingly affected by ethanol when the ethanol concentration increases. Ethanol interferes with the photo-oxidation reaction of sucrose and suppresses the sucrose signal because of a decrease in concentration of UV absorbing intermediate(s). The signal is monotonically decreasing with the amount of ethanol added. Ethanol disruption occurs in a narrower band than that of the sucrose peak (Fig. 1B): ethanol thus suppresses the signal corresponding to the center of the sucrose peak, but not the tail, creating a valley. Considering the Taylor Dispersion Analysis of pressure mobilization [22], this means that ethanol diffuses faster than sucrose in NaOH 130 mM, which is indeed expected from the difference of sizes of the molecules: ethanol diffuses faster than glucose [23], which in turn diffuses faster than sucrose [24]. If sucrose is placed in the mobile phase, then ethanol can be detected indirectly as a negative peak (Fig. 1B). The (negative) peak is then Gaussian confirming that the unusual peak shape in direct detection is due to the difference of diffusion coefficients of the ethanol and the carbohydrate. The peak shape is of importance for determining the