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Chromatographic Separations in Sugar Analysis and Processes

Fast detection of anionic components in sugar and wine samples using a novel device based on capillary zone electrophoresis

Brian O'Flaherty, Wei-Ping Yang, S. Sengupta, A.L. Cholli *

Center for Advanced Materials, University of Massachusetts-Lowell, One University Avenue, Lowell, MA 01854, USA

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Abstract

The quality of sugar and wine are dependent on the processing steps that are involved to remove extraneous components of starting materials, sugar canes, sugar beets and grapes. Many of the residual components present in the final product such as organic acids (malate, oxalate, formate, acetate, pyroglutamate, etc.) and inorganic ions (nitrate, nitrite, chloride, sulfate, sodium, potassium, calcium, etc.) may influence on the quality. There is a need for the development of new devices that can detect these multiple species in near-real time so that each of the processing steps can be monitored effectively. Preliminary data are presented here on the applicability of such a near real-time detection device that is based on the principles of capillary zone electrophoresis to monitor organic acid and anionic impurities in white sugar and wine samples at concentration levels that are required to meet national and international standards. Applicability of this device to monitor water pollutants is also discussed.

Keywords: Sugar; Wine; Anionic components; Capillary zone electrophoresis

1. Introduction

The quality of a refined sugar is dependent on many factors that influence during the entire production process. Some of the factors that influence the quality of the finished product are the sugar beet or sugar cane produce as well as extraction and processing steps involved in making white sugar as a final product. The amount of non-sugar impurities left in the final product determines the quality of sugars. The most common sugar impurities include moisture, invert, ash, colorant molecules, organic acids, inorganic anions and cations, and metal ions (Bruijn & Bout, 1999; Godshall, 1999). Excessive presence of each one of these impurities can cause detrimental quality defects (Godshall, 1999). To adhere to the national and international specifications on these non-sugar impurity contents, it is desirable to have sensors or devices that can monitor most of these impurities in near-real time either during extraction and processing stages or at the final stage of the sugar or wine production.

The quality of standards for white sugar, for example, is evaluated in terms of so- called 'European Points' for the European Union (Magne & Mathlouthi, 1998). According to this point system, there are at least three grades of white sugar to differentiate the quality of sugar samples: grade 1 for sugars with eight points or less, grade 2 for sugars with 8–22 points, and finally grade 3 with points more than 22 (Magne & Mathlouthi, 1998). These points are given to sugars based on tests performed in three categories: the visual appearance using Braunschweig color types, the solution color at a wavelength of 420 nm, and the ash content (Magne & Mathlouthi, 1998). In a similar way, other countries and organizations have their own specifications and standards to grade the quality of sugar.

As the specifications become more demanding, it has become essential to use modern analytical techniques and procedures to characterize non-sugar content in sugars. There are various analytical techniques that have been used to detect sugar species (Bruijn & Bout, 1999). Each of these techniques provides selective information on certain impurities. For complete analysis of one sample, it may be required to subject a sample to several tests using a wide variety of analytical techniques and

* Corresponding author.
E-mail address: ashok_cholli@uml.edu (A.L. Cholli).

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procedures. This requires a laboratory based multiple analytical instruments to perform different tests and procedures. For such laboratory based test facilities, it may be cost prohibitive to maintain and operate with several person-hours for (1) maintenance and calibration of analytical instruments, (2) method development, and (3) sample testing. In addition, some of these instruments may take several minutes to hours to complete one test. For example, it may take as much as 40 min to 1 h to complete a test for detecting about 12 sugar species containing organic acids and some ionic species using ion chromatography (Magne & Mathlouthi, 1998).

Ideally, it is desirable to have one device that can be customized to rapidly detect multiple sugar impurities at the concentration level required by the sugar industries to meet national and international standards as well customer specifications. Our research effort is focused on the development of such devices for rapid sugar analysis that can detect several cationic, anionic, and organic acid impurities in sugar samples in near-real time at concentration levels required by the sugar and wine industries.

2. Suitability of capillary zone electrophoresis technique for sugar analysis

The scope of the present work is to show the application of a compact device that is developed on the principles of capillary zone electrophoresis (CZE). The emerging technology of capillary electrophoresis offers a quick, sensitive, economic and reliable method for monitoring sugar impurities. Since its debut, this method appears to be viable alternative to ion chromatography with number of advantages over it. The research work in the past 5–10 years has demonstrated that modern electrophoresis offers simplicity, greater separation efficiency, unique selectivity and high degree of matrix independence (Khaledi, 1998). The amount of sugar sample required for detecting its ionic impurities is a small fraction of one-drop sugar solution in water (approximately 40–50 nl) and small usage of chemical reagents per analysis makes it as an environmentally friendly device. The instrument typically consists of a capillary tube made of fused silica with an inner diameter of 50–100 μm and length of 10–100 cm, filled with suitable electrolyte (buffer) solution. Both ends are immersed reservoirs containing the same buffer, as sketched in Fig. 1. The sugar solution to be tested for its impurities can be loaded onto the capillary by one of the three methods commonly used today: (1) gravity injection; (2) hydrodynamic (pressure) injection; and (3) electrokinetic injection (Khaledi, 1998; Landers, 1994). Analytes will migrate and separated in the capillary when a high voltage (10–20 kV) is applied across the capillary. This is accomplished by applying the voltage to electrodes dipped into the reservoirs. The migration velocity of a specific ionic impurity of the sugar sample is dependent on many factors including (1) its electrophoretic mobility that is dependent on ion's charge/mass ratio, viscosity, pH and concentration of the buffer, among the other factors, and (2) bulk electroosmotic velocity of the buffer. Both positive and negative ionic species can be detected in a single run if there is a large electroosmotic velocity (Chien & Burgi, 1992). The common detection method is usually an indirect ultraviolet spectroscopic method, where the migrating ions cause a vacancy in the buffer containing UV absorbing chromophores. The light source and detection assembly is normally located close to one end of the capillary while the other end is normally used for sample injection.

Preliminary work has been presented here to demonstrate the applicability and suitability of such a device in detecting cationic and anionic inorganic and organic species in water, sugar and wine samples.

3. Experimental

3.1. Chemicals

All chemicals were of analytical grade, and were purchased from either Aldrich (Milwaukee, WI, USA) or Sigma (St. Louis, MO, USA). All solutions were prepared with high purity Millipore Milli-Q water (18 MΩ cm). All solutions were filtered through a 0.45 μm membrane filter.

The stock solutions containing 270 mM sodium chromate, 20mM cetyltrimethylammonium bromide (CTAB), and 99.9% acetonitrile were prepared. The buffer was prepared by diluting 0.7 ml of 270 mM

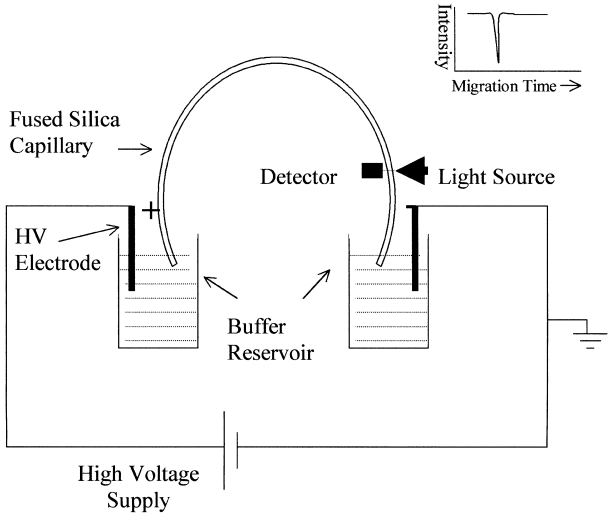

Fig. 1. Sketch showing the operating principles of the device based on CZE.

sodium chromate, 3.75 ml of 20 mM CTAB, and 1.2 ml acetonitrile to 30 ml total volume. The pH of the buffer was adjusted by the addition of 100 mM NaOH. Fresh background electrolyte solution was prepared daily.

3.2. Instrumentation

All the data were collected using a home-built device equipped with a high voltage power supply, indirect UV detection scheme consisting of a UV source and detectors with band pass filters operating either at $\lambda = 214$ or 254 nm. The instrument was interfaced with a PC through an analog-to-digital converter (ADC) board for data acquisition and collected data were processed using commercially available software (Cholli et al., 2000).

3.3. Capillaries and capillary washing

A 17 cm × 50 μm I.D fused-silica capillary tubing coated with polyimide (Polymicro Technologies, Phoenix, AZ, USA) was used in this device. The polymer coating was burned off at 4 cm from one end of the capillary, to form the detection window.

The capillary was rinsed with 0.2 M NaOH (30 min), deionized water (5 min) and then with separation buffer (30 min). Samples were injected by electrokinetic injection by applying a 0.5 kV for 6 s.

4. Results and discussion

A typical response of the CZE device for a sample containing a mixture of standard samples is shown in Fig. 2. These are typical organic acids and anions present in the samples of interest in the present study. The concentration of each species in this sample was 1 ppm. In this electropherogram all the nine species are separated and able to detect in < 1 min of analysis time. Actual time difference between the first peak (chloride ion) and the last migrating ion (pyroglutamic acid) is approximately 30 s in Fig. 2. It appears from Fig. 2 that the resolution, separation efficiency and sensitivity are adequate for the sugar and wine industries to meet the specifications. Our recent data show that sensitivity can be improved further by optimizing the detection scheme to detect these ionic species in the parts-per-billion (ppb) range. There are many factors, apart from the physical design of the device, which can influence the resolution and sensitivity for detecting sugar and wine impurities. Some of the factors that influence on the quality of data are composition of the BGE, detection mode, buffer pH, temperature, electroosmotic flow (EOF) modifiers and their concentration, among other experimental conditions (Kaniansky, Masar, Marak, & Bodor, 1999; Khaledi, 1998; Landers, 1994).

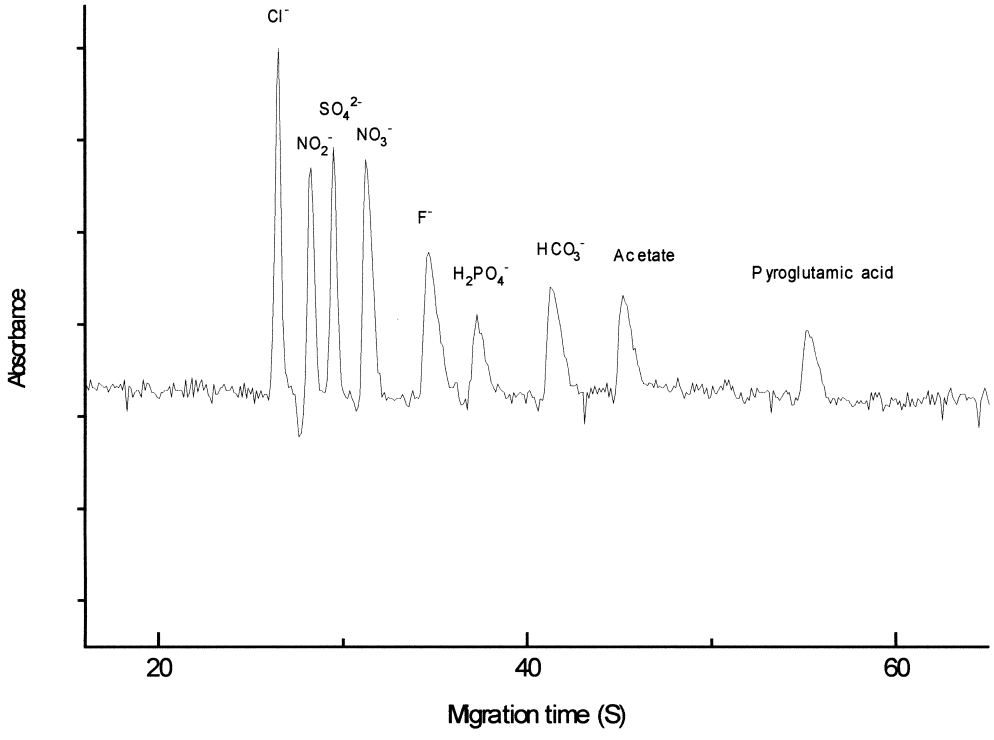

Fig. 2. Response of the device (Electropherogram) showing the detection of nine anionic and organic acid spieces in less than one minute.

4.1. Assessment of analytical response

Preliminary evaluations of the technique are presented here to include linearity, precision and detection limits. The response of the CZE device for inorganic anions that are shown in Fig. 2 were correlated with the concentration of these analytes in the sample injected. The plot shown in Fig. 3 shows a linear response for the concentration over a range of 1–50 parts-per-million (ppm). This response has been extended to 1–100 ppm. An internal standard was used during this study. Each data point in Fig. 3 is an average of at least three repeated injections. The regression equation with their correlation coefficient (R) and limit of detection for the species studied in Fig. 2 are tabulated in Table 1. The data shown in Table 1 suggests that the correlations are >0.999 and the limit of detection (LOD) are in the ppb range. With additional improvements in the detection scheme and analysis method, it is possible that LOD can significantly be lowered to the ppt (parts-per-trillion) range. The LOD was evaluated using the equation described by Mrestani, Neubert, Haertl, and Wohlrab (1997).

5. Applications to detection of impurities in water, sugar and wine samples

5.1. Analysis of water sample

Water is a natural source that is used for drinking, agricultural irrigation, industrial applications, aquatic life and recreational activities. A recent report submitted to US Congress (Report to Congress, 1998) suggests that rivers, lakes, reservoirs, and ground waters are significantly contaminated by pollution that affects human life. Polluted water causes serious damages to human health. It is known that some of the contaminants are sources for cancer, leukemia, and other life threatening diseases. For example, extensive application of fertilizers in agriculture farming, residential lawns, and golf courses may lead to ground water

Table 1
Calibration data of the Device for anions

Ionic species	Linear equation $Y=a+bX$	Correlation coefficients (R)	Limit of detection (LOD), ppm
Chloride	$Y=0.1268+0.4040X$	0.9994	0.08
Nitrite	$Y=0.0128+0.2748X$	0.9996	0.3
Sulfate	$Y=0.0932+0.3026$	0.9993	0.1
Nitrate	$Y=0.00736+0.2159X$	0.9995	0.1
Fluoride	$Y=0.0437+0.7720$	0.9996	0.07
Bicarbonate	$Y=0.2143+0.1864X$	0.9996	0.3

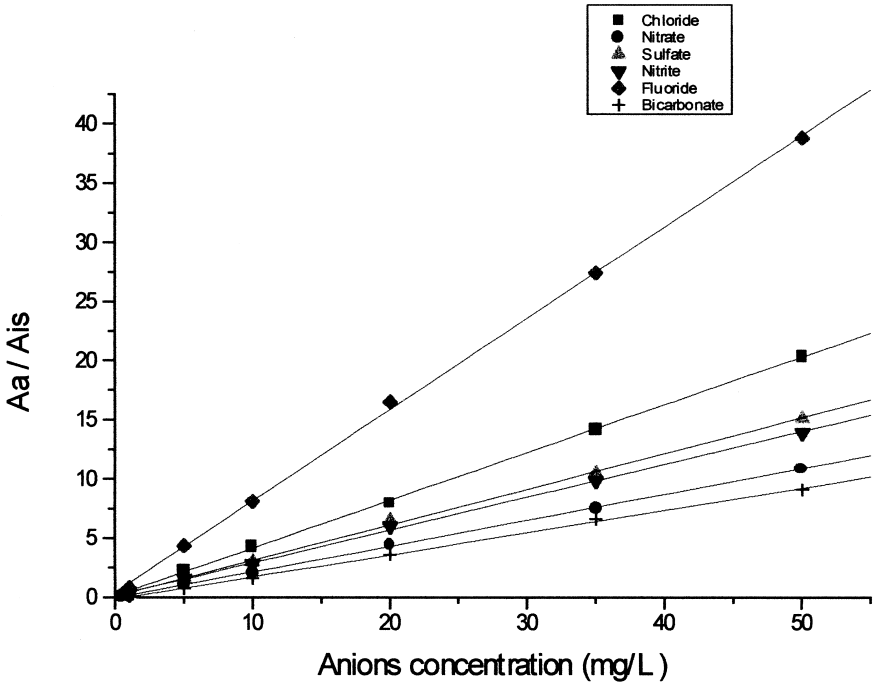

Fig. 3. Calibration data showing a linear response in the 1–50 ppm range for various anionic species.

contamination underneath the soil surface. Nitrate dissolves readily into water and can be easily transported from soil surface to underground water resource. An exposure of 10 ppm of nitrite from contamination activities may cause blue-baby syndrome (methemoglobinemia) in humans. Infants under three months are more susceptible to this syndrome than adults (Sadecka & Polonsky, 1999). This syndrome creates a situation where human body is unable to fix the oxygen level in blood. Such health concern associated with polluted water necessitates the need for monitoring of water quality

Fig. 4 illustrates the application of the device for monitoring specific anions in water. The electropherogram A for the tap water is from the city of Lowell, MA and was recorded without the presence of an internal standard while b was recorded with the presence of an internal standard. It shows the presence of major anionic species present in the tap water. The testing was completed in less than 1 min. Using the calibration data presented in Fig. 3 it is possible to estimate their concentrations in water samples. It is clearly noticeable that a very high concentration chloride anions (35 ppm) in the city water, while other species are at much lower concentrations, nitrite (0.4 ppm), sulfate (8.4 ppm), nitrate (1.6 ppm), fluoride (0.7 ppm) bicarbonate (17.4 ppm). Analysis of waters samples from different sources like bottled waters and river water indicate that these concentrations vary significantly from one sample to the other (Yang, O'Flaherty & Cholli, 2001). These measurements suggest that the device and approach presented here is fast and has ability to detect anionic species present in water in <1 min.

5.2. Sugar and wine analysis

In the production of white sugar either from sugar cane or beet, there is a need for the analysis of components in both raw and processed products. The major steps involved are the removal of extraneous components that adversely affect the final quality of the sugar (Godshall, 1999). If the processing conditions were ideal, one would expect the final refined product to be sucrose. If the components other than sucrose are present as minor constituents or impurities in the final sugar product even after subjecting the material to a multi-step processing, they may contribute to the undesired properties (Godshall, 1999).

Here we show the applicability of our device for the analysis of organic acids and anionic components in the white sugar samples; the constituents of these samples have been characterized by traditional techniques that are normally used in the sugar industries (Bruijn & Bout, 1999). Their analysis for organic acids and anions is presented in Table 2. In Fig. 5, the electropherogram of white sugar-1 sample is shown. It shows *simultaneous* detection of both organic acids and anions in *a single run* of analysis. The time to complete one analysis was again <60 s. In (Fig. 5), all the peaks for the ions of interest were detected. These are preliminary data and were collected without optimizing the conditions for the sugar analysis. The signal-to-noise ratio is lower compared with the data collected on the other samples. This

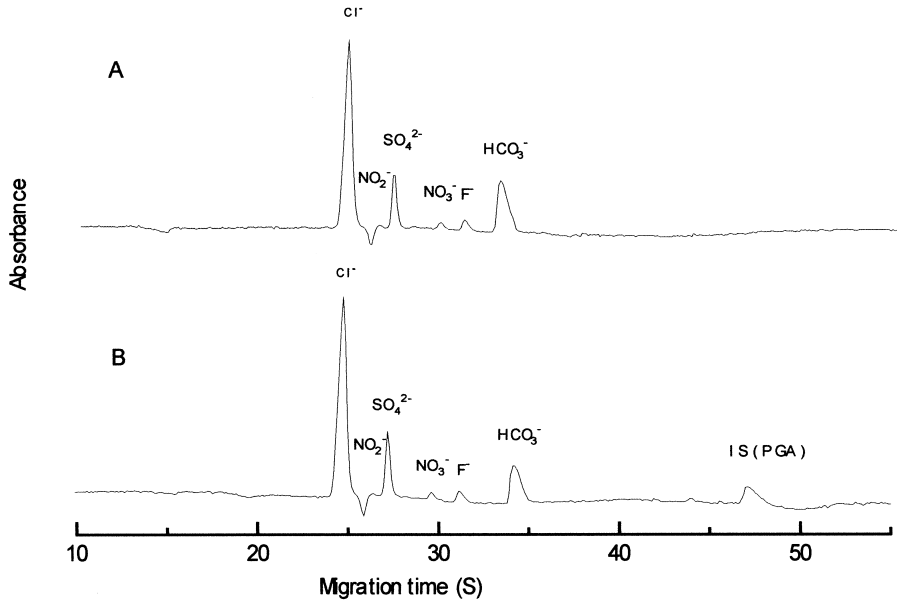

Fig. 4. Detection of anionic species in the tap water of the city of Lowell, MA. (A) Electropherogram was collected without adding an internal standard, (B) electropherogram with an internal standard.

is mainly because of an excess amount (15 ppm) of internal standard was added while analyzing this set of sugar samples. The relative intensities of peaks suggest that sulfate is in higher concentration (6.5 ppm) compared with chloride (3.3 ppm) and nitrate (3.8 ppm) ions. The presence of these ions is originally from the sugar beets itself and not from the extraction procedures, since these are inert ions pass through without any change (Bruijn & Bout, 1999). A higher concentration of chloride is expected if calcium chloride is added to the extractor for the improvement of the process ability of the pulp. In such cases, as a result of it, higher concentration of chloride in white sugar samples is expected (Bruijn & Bout, 1999). The presence of higher concentration of sulfate in Fig. 5 is most likely due to the addition of sulfuric acid/or gypsum during the pulp processing and at the juice extraction stage to control the level of pH (Bruijn & Bout, 1999). A low level of nitrite is expected in white sugars in the processes where microbial activities are effectively suppressed. It is known that microorganisms with nitrate reductase activities can reduce nitrate into nitrite during juice purification process. Other possible source for the presence of nitrate and nitrite may be from the raw materials. Fig. 5 shows the presence of nitrite but at very insignificant level (almost noise level) in the sugar sample studied.

Table 2
Anions and organic acid impurities in the sugar samples analyzed

Component	White sugar		
	CSM-1	CSM-2	CSM-3
Organic acids (mg/kg, ppm)			
Oxalate	6.2	0.3	0.9
Citrate	0.0	0.0	0.0
Malate	2.4	2.8	2.4
Lactate	9.2	9.2	11.2
Formate	1.1	2.0	2.0
Acetate	2.6	2.2	2.9
Pyroglutamate	15.6	15.2	13.5
Inorganic anions			
Chloride	3.3	3.7	4.4
Nitrite	0.1	0.4	0.6
Phosphate	0.0	0.0	0.0
Sulfite	2.4	2.4	3.6
Sulfate	6.5	4.6	3.9

Experimental data shown in Fig. 5 qualitatively agrees also with the data shown in Table 2 for organic acids present in white sugar samples. The presence of acetate, lactate, and formate are probably from the raw materials, whereas pyroglutamate is due to saponifica-

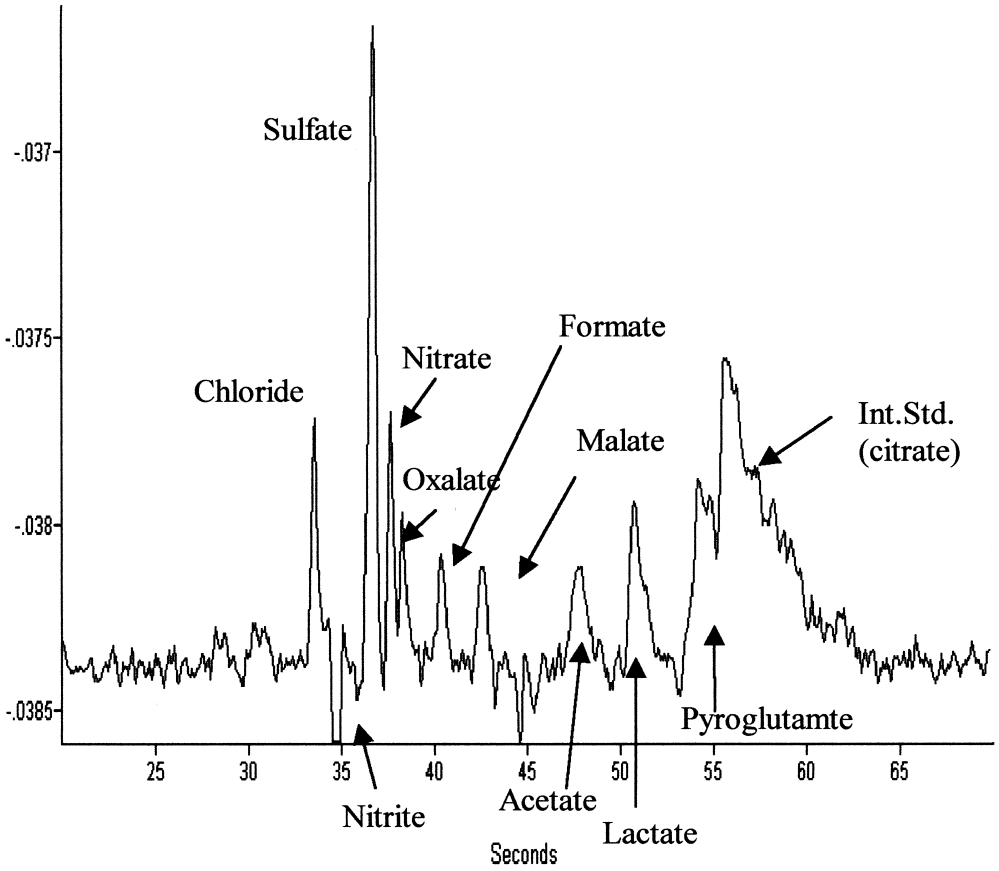

Fig. 5. Fast detection of impurities in the white sugar sample. Organic acid and anionic type species are detected in a single run with an experimental time less than 1 min. See Table 1 for their concentrations (in ppm).

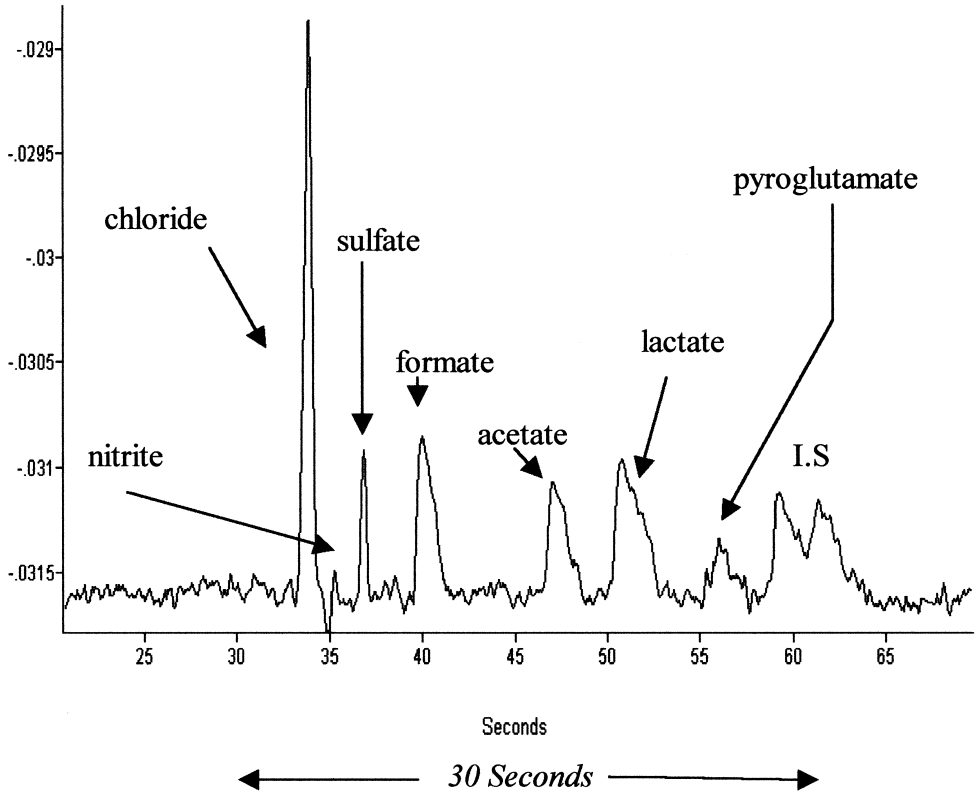

Fig. 6. Fast detection of impurities in a white sugar sample made from sugar cane (commerical product from Domino).

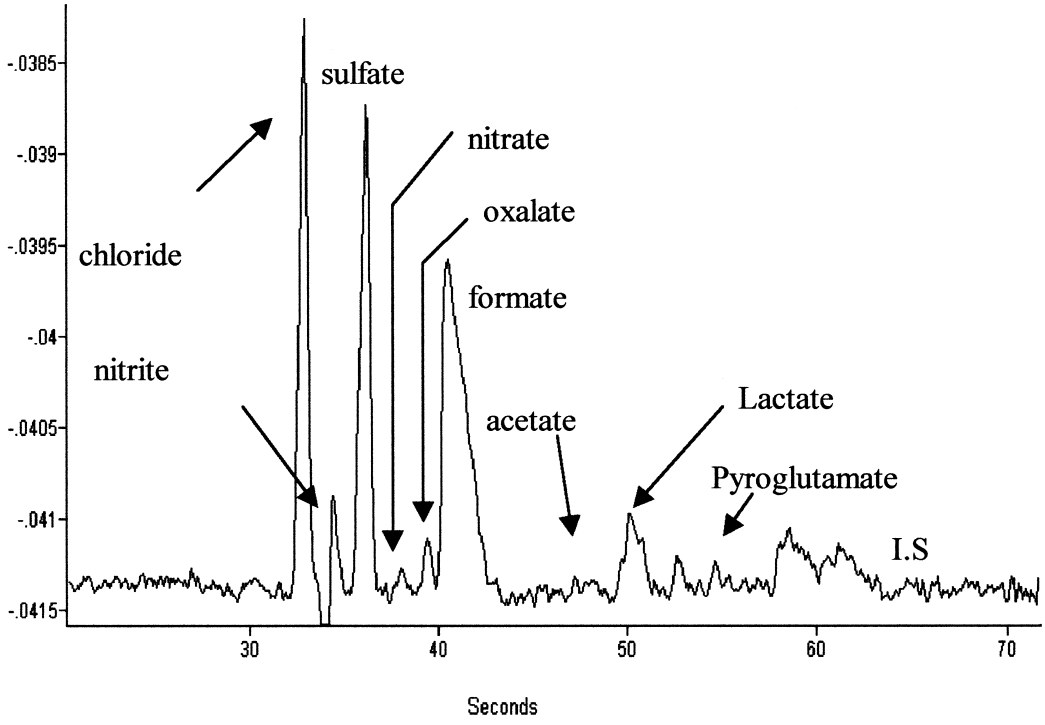

Fig. 7. Detection and identification of impurities of the organic acid and anionic type species in a Californian wine (Chardonnay, Ernest and Julio, Gallo).

tion of glutamate during the juice purification. The concentration of pyroglutame in Fig. 5 is significantly higher compared with other organic acid impurities in white sugar. A wide variety of other sugar samples have been analyzed using this device. Fig. 6 shows the presence of organic acids and anions in a commercially available American sugar (Domino, White sugar). Comparison of data in Figs. 5 and 6 suggests that the concentration of impurities in white sugars from cane and beet are different. This may be due to different extraction and processing conditions in these two cases.

The design of the instrument can be modified to analyze cationic impurities in the sugar samples and the results will be reported later.

In a similar way, it is possible to analyze wines for monitoring the presence of impurities either during the processing stage or at the final stage to monitor the quality of the finished product. In Fig. 7 the response of the device for a Californian wine (Chardonnay, Ernest and Julio, Gallo) sample is shown with the same experimental conditions that were set for analyzing sugar samples. In comparison with sugar samples, the concentration levels of organic and anionic impurities are at different levels. This is expected since wine processing, ingredients and raw materials are not the same compared with sugar processing. However, it is interesting to note that in both sets of samples, similar kinds of impurities were observed. Analysis of different labels and kinds of wine suggest that the device described here is suitable to monitor simultaneously both organic acids and inorganic anions in wine samples to monitor the extraction and processing conditions, and also the quality of finished product. Work is in progress on the method development for the device to quantify and analyze impurities including cations, and also to assess the potential limiting factors for such a device.

6. Summary

Preliminary data from our sensing device suggest that it is possible to monitor multiple impurities in water, sugar and wine samples in near-real time. The promising aspect of the device is that it can detect organic acids and ions in a single run and the total analysis time is < 1 min. It appears that the sensitivity of the device is more than adequate for rapid analysis of sugar and wine samples to meet national and international standards.

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