Analysis of Organic Acids and Inorganic Anions in Different Types of Beer Using Capillary Zone Electrophoresis

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Two methods for the investigation of different types of beer by capillary zone electrophoresis are presented. The first separation system described in this work allows the quantitative analysis of beers with respect to their contents of low molecular mass anionic components using indirect ultraviolet detection as well as conductivity detection, providing relative standard deviation between 0.5 and 6.6% for the peak areas and excellent limits of detection (LOD) ranging from 0.02 mg L^{-1} for chloride to 0.41 mg L^{-1} for phosphate. The second method offers the possibility of fast determination of amino acids in beer samples without the necessity of any sample pretreatment. LODs obtained for the investigated solutes were found to be strongly dependent on their spectroscopical properties and in the range of 0.5−50 mg L^{-1}. Despite this restriction, this analytical method can be regarded as a suitable tool for the screening of beers with respect to their amino acid patterns.

Keywords: *Capillary zone electrophoresis; beer samples; low molecular mass anionic compounds; amino acids*

INTRODUCTION

Capillary zone electrophoresis (CZE) is becoming an attractive alternative to chromatographic techniques and has gained increasing importance in the analysis of low molecular mass organic acids (Klampfl and Buchberger, 1997) and inorganic ions (Jackson and Haddad, 1993) in a variety of real samples within the past years. However, only a few publications deal with the determination of these solutes in beverages and especially in beer samples by CZE (Cancalon, 1997).

Among the applications reported so far, citrus juices have been analyzed by CZE with respect to their amino acid composition using a pH 9.3 Borax carrier electrolyte and direct ultraviolet (UV) detection at wavelengths below 210 nm (Cancalon, 1993; Cancalon and Bryan, 1993) as well as with respect to their content of minor inorganic anions using a chromate-based running buffer and indirect UV detection (Swallow and Low, 1994). Employing the latter detection technique, organic acids have been determined in sport drinks and fruit juices using trimellitic acid as background electrolyte (Wu et al., 1995). Separation of inorganic anions and organic acids in combination with indirect UV detection was possible for apple juice and wine samples with pyromellitic acid serving as running buffer (Arellano et al., 1997) and for wine samples by employing a chromate-based electrolyte (Dedieu et al., 1994).

Concerning beer samples, organic acids and inorganic anions have been investigated by means of 2,6-pyridinedicarboxylic acid as carrier electrolyte (Soga and Ross, 1997) and indirect UV detection at 195 nm. Using direct UV detection at 185 nm and a phosphate-based running buffer including octanesulfonic acid to enhance the quality of the separation, amino acids have been analyzed in a number of beer samples (Klampfl et al., 1998). Low molecular mass anionic compounds have also been analyzed in fermented beverages by employing conductivity detection and a running buffer based on histidine (His) and morpholinoethanesulfonic acid (MES) (Jones, 1998).

This work presents investigations on the quantitative analysis of beer samples with respect to their content of organic acids (amino acids, carboxylic acids) and inorganic anions by CZE using the best suited system (carrier electrolyte, method of detection) for the determination of each type of analyte.

MATERIALS AND METHODS

Apparatus. For the analysis of inorganic anions and carboxylic acids a Crystal 310 CZE instrument (Thermo Bioanalysis, Santa Fe, CA) equipped with a Crystal 1000 conductivity detector (Thermo Bioanalysis) and a fixed-wavelength UV detector operated at 254 nm (Waters, Milford, MA) was used. Amino acids were determined by means of a Quanta 4000 CE instrument (Waters) equipped with a fixed-wavelength UV detector (mercury lamp operated at 185 nm). Data collection was performed with an HP 3359 data acquisition system (Hewlett-Packard, Palo Alto, CA). The Thermo CE instrument was used in combination with ConCap I fused-silica capillaries (Thermo Bioanalysis) with an inner diameter of 50 μm, whereas in the case of the Waters device fused-silica capillaries from Polymicro Technologies (Phoenix, AZ) were used.

Carrier Electrolytes. For the determination of inorganic anions and carboxylic acids a running buffer made from 7.5 mM 4-aminobenzoic acid (p-AB) with a pH of 5.75 adjusted by the addition of histidine (His) was employed. To this background electrolyte was added 0.12 mM tetradecyltrimethylammonium bromide (TTAB) for reversal of the electroendoosmotic flow (EOF). In the

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case of amino acid analysis a carrier electrolyte consisting of 10 mM NaH_2PO_4 and 30 mM octanesulfonic acid (pH 2.36) adjusted by the addition of phosphoric acid was used. High-purity (18 MΩ) water obtained from a Milli-Q system (Millipore, Marlborough, MA) was employed for the preparation of all solutions.

Procedures. New capillaries were treated with a 0.5 M NaOH solution and water for 30 min before use. Prior to each analysis the capillary was flushed with running buffer for 3 min. Injection was performed in a hydrodynamic mode at the cathodic side for the analysis of inorganic anions and carboxylic acids and at the anodic side when amino acids were determined.

Beer Samples and Reference Compounds. Standard solutions were made by dissolving the appropriate salts or carboxylic acids (purity > 99%) in high-purity water. Beer samples were diluted with water 10-fold for the determination of inorganic anions and carboxylic acids or 4-fold in the case of amino acid analysis, respectively. Prior to analysis they were degassed for 15 min in an ultrasonic bath.

RESULTS AND DISCUSSION

Determination of Inorganic Anions and Carboxylic Acids. In general, inorganic anions and low molecular mass carboxylic acids can be analyzed by CZE combined with by indirect UV detection (UVD) using a UV-absorbing carrier electrolyte, by direct UVD in the case of UV-absorbing solutes, or by conductivity detection (CD). Comparison of these techniques reveals that UVD as well as CD can be regarded as suitable for the analysis of low molecular mass anionic compounds in general, whereas the applicability of direct UVD is often restricted. For the determination of inorganic and organic anions in different types of beer, it proved to be advantageous to use CZE with CD in series with UVD at 254 nm to profit from the benefits of both methods of detection. To fulfill the requirements of both, UVD as well as CD, the employed carrier electrolyte should exhibit low conductivity and must contain a UV-absorbing anion to show compatibility with both detection techniques. Therefore, a running buffer made of 7.5 p-AB and 0.12 mM TTAB (for EOF reversal) and adjusted to pH 5.75 by the addition of His was used for analysis. The electropherograms obtained for a diluted lager beer are depicted in Figures 1 (UVD) and 2 (CD). As can be seen from these plots, CD proved to be more sensitive for the faster migrating anions, whereas UVD was found to be superior for analytes with mobilities similar to that of p-AB. By the use of both detectors simultaneously, it was possible to perform quantitation using the best suited method of detection for each type of solute, namely, CD for the range chloride to succinate (peaks 1−7) and UVD from pyruvate to pyroglutamate (peaks 8−12). This approach resulted in excellent limits of detection (LODs) for all analytes of interest. They were found to be in the range of 0.02 mg L^{-1} for chloride using CD and 0.41 mg L^{-1} for phosphate using UVD. To enhance the reproducibility of the quantitation procedure, the beer samples were spiked with two internal standards. In combination with CD, solute concentrations were determined relative to chlorate (I.S. 1), whereas 5-chlorovalerate (I.S. 2) was used for this purpose when UVD was employed. The upper part of Table 1 lists the amount of the selected anionic solutes found in the different beer samples under investigation. As can be seen from these data, excellent relative

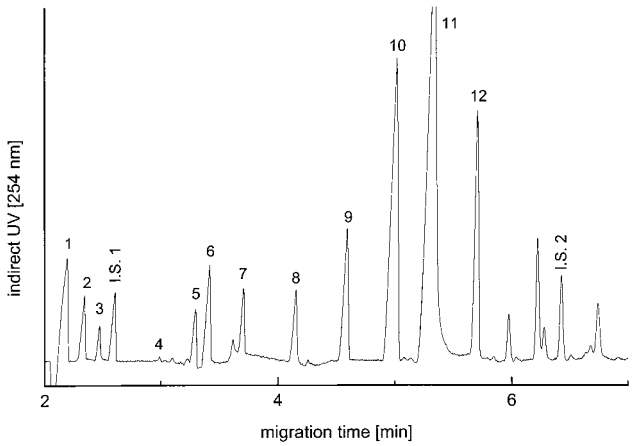

Figure 1. Electropherogram obtained for a diluted lager beer sample using indirect UV detection at 254 nm. Peaks: 1 = chloride; 2 = sulfate; 3 = oxalate; 4 = formate; 5 = malate; 6 = citrate; 7 = succinate; 8 = pyruvate; 9 = acetate; 10 = lactate; 11 = phosphate; 12 = pyroglutamate. Carrier electrolyte, 7.5 mM 4-aminobenzoic acid containing 0.12 mM TTAB, pH adjusted to 5.75 with His; applied voltage, −30 kV; injection, 25 mbar for 0.2 min; capillary, 48 cm effective length × 50 μm i.d.

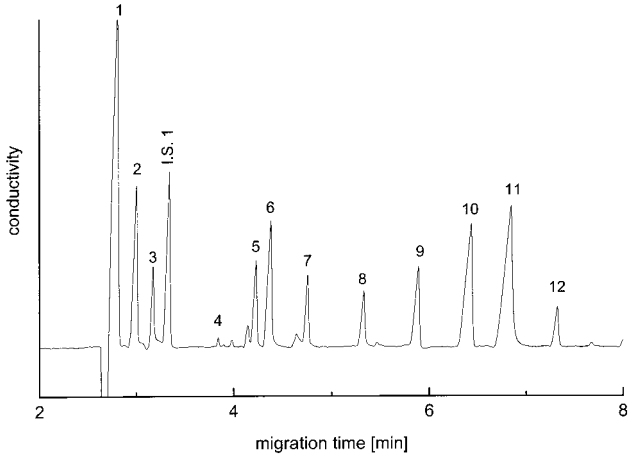

Figure 2. Electropherogram obtained for a diluted lager beer sample using conductivity detection. For conditions and peaks, see Figure 3 except capillary, 60 cm effective length × 50 μm i.d.

standard deviation (RSD) values could be obtained for most of the analytes. Only the value for formate in the white beer sample is affected with a somewhat higher RSD because it was close to the limit of quantification. Regarding the selected beer samples, differences in the concentration patterns of the analytes could be found that are related to the source of water used for the fermentation process as well as variations in the brewing procedure. Whereas high amounts of phosphate and lactate were detected in all beers, analytes such as oxalate, formate, and succinate were present only in lower quantities. In the case of the nonalcoholic beer, notably low concentrations of succinate, pyruvate, and pyroglutamate compared to the other beer samples under investigation were found.

Analysis of Amino Acids. Besides proteins and peptides, free amino acids can also be found in beer, mainly originating from the malt component of this beverage (Caralambous, 1981). Because of their zwitterionic nature, amino acids can be separated in their anionic or cationic form. We analyzed free amino acids in different types of beer without any derivatization step

Table 1. Content (Milligrams per Liter) of Inorganic Anions and Organic Acids in Different Beers[a]

	Chinese rice beer	white beer	nonalcoholic beer	lager beer
		Inorganic Anions		
chloride	181 ± 1.7	215 ± 1.5	247 ± 2.6	101 ± 1.1
sulfate	191 ± 2.2	102 ± 2.7	128 ± 3.3	63 ± 0.8
phosphate	548 ± 0.5	961 ± 1.3	387 ± 2.3	917 ± 1.5
		Carboxylic Acids		
oxalate	17 ± 0.7	9 ± 3.1	10 ± 4.1	31 ± 1.3
formate	4 ± 3.6	3 ± 6.6	6 ± 1.4	nd
malate	40 ± 2.7	87 ± 0.6	31 ± 1.3	68 ± 1.4
citrate	193 ± 2.4	171 ± 1.5	137 ± 2.7	178 ± 1.1
succinate	42 ± 2.7	82 ± 2.6	18 ± 2.1	49 ± 1.3
pyruvate	82 ± 2.9	84 ± 1.3	29 ± 4.2	108 ± 3.5
acetate	43 ± 0.9	128 ± 0.7	64 ± 1.3	105 ± 1.1
lactate	351 ± 2.5	508 ± 1.0	349 ± 2.6	556 ± 0.7
pyroglutamate	279 ± 1.1	218 ± 1.5	123 ± 1.6	258 ± 1.1
		Amino Acids		
lysine	588 ± 3.3	nd	282 ± 1.2	124 ± 12.8
histidine	nd	nd	30 ± 7.2	31 ± 6.5
arginine	120 ± 5.5	76 ± 2.3	91 ± 2.4	42 ± 7.8
glycine	140 ± 15.9	138 ± 8.3	109 ± 6.4	68 ± 4.7
alanine	193 ± 8.3	nd	nd	110 ± 12.6
tryptophan	25 ± 2.3	49 ± 1.0	48 ± 7.7	36 ± 11.1
phenylalanine	55 ± 2.6	95 ± 1.4	94 ± 0.4	58 ± 7.5
tyrosine	71 ± 2.6	419 ± 2.2	252 ± 1.6	215 ± 5.8
proline	620 ± 1.6	546 ± 1.3	246 ± 3.4	296 ± 7.6

[a] nd, not detected; values represent means (± relative standard deviation %) of three determinations.

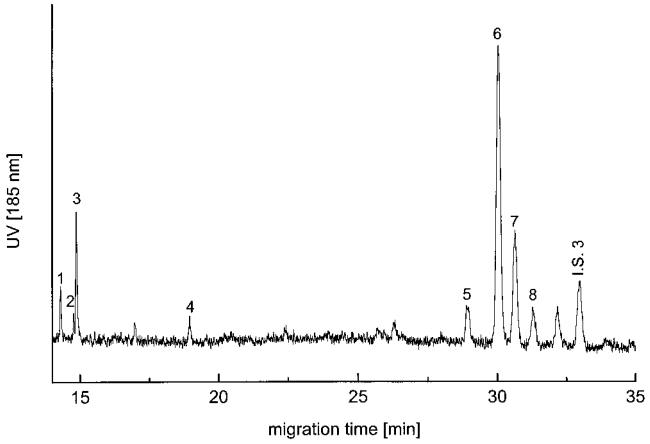

Figure 3. Electropherogram showing the amino acid pattern of a nonalcoholic beer sample. Peaks: 1 = lysine; 2 = histidine; 3 = arginine; 4 = glycine; 5 = tryptophan; 6 = phenylalanine; 7 = tyrosine; 8 = proline. Carrier electrolyte, 10 mM NaH_2PO_4 containing 30 mM octanesulfonic acid, pH 2.36; applied voltage, 30 kV; injection, 10 mbar for 0.25 min; capillary, 100 cm effective length × 50 μm i.d.; direct UV detection at 185 nm.

using a 10 mM NaH_2PO_4 carrier electrolyte containing 30 mM octanesulfonic acid and adjusted to pH 2.36 by the addition of phosphoric acid in combination with direct UVD at 185 nm. Under these conditions the selected amino acids can be regarded as cations. Previous investigations revealed that the addition of larger amounts of sulfonic acids was useful to improve the resolution of a number of adjacent peaks (Thornton et al., 1997). In Figure 3 the electropherogram obtained for a diluted nonalcoholic beer using the separation conditions mentioned above is depicted. In this sample eight peaks could be identified. Cysteine was added as internal standard (I.S. 3) to correct migration time shifts as well as errors related to the injection procedure. Quantitative analysis of the beer samples was performed by external calibration with standard mixtures covering the concentration range of interest. The LODs as well as RSDs are strongly dependent on the spectroscopic properties of the corresponding analyte. RSD values obtained for standard mixtures of the solutes at concentration levels similar to those present in the samples were <2% for tryptophan (an aromatic amino acid showing strong UV absorbance at the selected detection wavelength) and 3.5−9% for aliphatic amino acids providing only weak UV absorbance. LODs ranged from 0.5 mg L^{-1} for tryptophan and phenylalanine to 10−50 mg L^{-1} for aliphatic analytes. Regarding the quantitative analysis of the selected beers, significantly different amino acid patterns could be found, as can be seen from the data shown in the lower part of Table 1. Relatively high amounts of proline and tyrosine (except in the rice beer) could be detected in all samples under investigation, whereas amino acids such as tryptophan or histidine (present only in the nonalcoholic beer and the lager beer) could be found only in minor concentrations.

Conclusion. The results presented in this work demonstrate that CZE is an attractive technique for the analysis of inorganic anions and organic acids in beer samples. By combination of CD and UVD notably low LODs and RSDs could be obtained for quantitative analysis of inorganic and organic anions with a wide range of different mobilities. Although the CZE method used for the determination of underivatized amino acids does not lead to LODs as low as those achieved by methods that include a derivatization step, this technique can be regarded as a suitable tool for the fast screening of the investigated samples with respect to their amino acid patterns because no sample pretreatment is required.

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