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Determination of low-molecular-mass organic acids in any type of beer samples by coelectroosmotic capillary electrophoresis

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Abstract

A separation and determination of a mixture of 19 low-molecular-mass organic acids usually present in beer samples was developed using coelectroosmotic capillary zone electrophoresis. A polycation (hexadimetrine bromide, HDB) has been added to the electrolyte, which dynamically coats the inner surface of the capillary and causes a fast anodic electroosmotic flow. The main factors affecting reversal of the EOF such as type of modifier and concentration and influence of organic solvents were studied. Three types of modifiers, two alkylammonium salts (cethyltrimethylammonium bromide and tetradecyltrimethylammonium bromide) and a polycation (HDB) were investigated. The composition of the running buffer results on a 25% 2-propanol, 0.001% HDB and 50 mM sodium phosphate. The different instrumental parameters affecting the capillary electrophoretic separation were also optimized resulting on a −15 kV voltage with a hydrodynamic injection for 7 s with a UV detection at 210 nm. The applicability of the present method has been demonstrated for the determination of organic acids in different beer samples.

Keywords: Beer; Organic acids; Coelectroosmotic capillary electrophoresis

1. Introduction

A great number of organic acids are present in beer which concentration depends on the raw materials, the brewhouse procedure, the yeast and fermentation conditions [1]. A wide variety of organic acids are produced during fermentation, some of which make important contributions to flavour [2]. So, organic acids are important beer constituents because of their effect on taste and shelf-life of beer, as a yardstick of whether fermentation is proceeding normally and as a means of distinguishing between different types of beers, which will permit to give indications regarding differences in the composition of the raw materials employed and in variations in brewing and fermentation techniques.

Capillary zone electrophoresis (CZE) is gradually gaining acceptance as an alternative and complementary technique to high-performance liquid chromatography (HPLC) for the food analysis. Principles advantages of CZE include, among others, high separation efficiency, improved selectivity, low operational cost and speed of analysis [3,4]. Specific requirements for the validation of CE methods have been published [5,6].

To anionic compounds the analysis time can be reduced by reversing the EOF. A principle known as coelectroosmotic CE has been used succesfully for the analysis of anionic compounds such as inorganic anions [7,8], phenolic compounds [9,10] or carboxylic acids [11]. Under such conditions, a negative power supply causes anionic compounds to migrate in the same direction as the electroosmotic flow. Adding cationic surfactants such as hydrophobic quaternary alkylammoniumm ions, to the carrier electrolyte, at concentrations below

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the critical micelle concentration (CMC), reverse the EOF by dynamically coating the inner capillary wall.

Actually, different works related to the determination of organic acids in beer by CE have been published [12–17].

The coelectroosmotic CZE analytical conditions established in this study were applied to determine the content of different organic acids in real commercial beer samples and demonstrate the possibilities of capillary electrophoretic methods for the analysis of beer samples as an interesting alternative to other separation techniques more widely established in the brewing industry.

2. Experimental

2.1. Chemicals

The organic acids were purchased from Sigma (USA). They are divided in two groups: aliphatic acids (ketoglutaric, fumaric, malic, mesaconic, oxalic, pyroglutamic, pyruvic and sorbic) and aromatic acids (4-aminobenzoic, benzoic, *p*-coumaric, ferulic, phthalic, gallic, 4-hydroxybenzoic, homovanillic, protocatechuic, sinapinic and syringic).

Sodium tetraborate (borax) and sodium phosphate were used as buffers and were obtained from Sigma (USA). Ethanol (EtOH) of 99.8% purity (UV, IR, HPLC) was purchased from Panreac (Spain).

The modifiers cetyltrimethylammonium bromide (CTAB), tetradecyltrimethyl ammonium bromide (TTAB) and hexadimetrine bromide (HDB) were purchased from Sigma (USA). The organic solvent used was 2-propanol obtained from Merck (Germany). The water used was obtained using the Milli-Q purification system of Millipore (Bedford, MA, USA).

2.2. Preparation of standard solutions

Stock standard solutions at different concentrations of a mixture containing all the analytes under study were prepared in doubly deionized water (oxalic and malic acid at 6000 mg/l; ketoglutaric and pyruvic acid at 2000 mg/l; pyroglutamic acid at 1000 mg/l; fumaric, mesaconic, phthalic, benzoic, sorbic, 4-aminobenzoic, 4-hydroxybenzoic, protocatechuic, gallic, *p*-coumaric, homovanillic, syringic, ferulic and sinapinic acid at 200 mg/l). Working standard solutions were prepared daily by dilution of these solutions with Milli-Q water and mixing with 5% EtOH. Stock aqueous solutions of CTAB, TTAB and HDB were prepared weekly at 10 mM and 0.05% (w/v), respectively.

2.3. Instrumentation

The experiment were performed on a P/ACE System MDQ capillary electrophoresis instrument (Bekman Coulter, Fullerton, CA, USA). A diode-array detection (DAD) system was used to detect the individual compounds at their optimum wavelengths and to identify them by comparing their UV spectra with those of the reference compounds. Data acquisition and processing were carried out with gold software installed in a personal computer. The system comprises a 0–30 kV high-voltage built in power supply, equipped with a diode array detector. All capillaries (fused silica) used were obtained from Beckman Instruments (Fullerton, CA, USA) and had an inner diameter of 75 μm, a total length of 57 cm and an effective separation length of 50 cm. The DAD system was positioned at 210 nm.

2.4. Methodology

The optimum separation conditions of CZE were performed at 50 mM sodium phosphate, pH 8, 0.001% HDB and 25% 2-propanol. Capillary tube was conditioned with 0.5 mol/l NaOH for 2 min, deionized water for 2 min and corresponding run buffer for another 5 min. Standards and samples were injected hydrodynamically for 7 s at 0.5 psi pressure injection and then the analytes were separated by applying a voltage of −15 kV and detected on-column at 210 nm.

Post-run, rinsing consisted of buffer for 5 min by applying both high voltage (30 kV) and pressure (20 psi; 1 psi = 6894.76 Pa) which results in separations with higher repeatability than by solely purging the capillary with electrolyte. The effect of purging and high voltage can be explained by a faster regeneration of the inner capillary surface and, consequently, a stable EOF.

2.5. Sample preparation

Seven different types of malt beers were facilitated from the company Grupo Cervezas Alhambra, S.L. These are: *Extra* (6.4, v/v), *Classic I* and *Classic II* (4.6%, v/v), *Special* and *Special black* (5.4%, v/v), *Light beer* (2%, v/v) and a *Non-alcoholic beer* (< 1%, v/v). The different beer samples needs only to be degassed and filtered through a 0.45 μm filter prior to the analysis and direct injection into the CE DAD system.

3. Results and discussion

For the selection of the analytes, different bibliographic sources were consulted [1,2,18,19] and different analysis of beer samples were made in order to select a group of organic acids that can be appear in any type of beer. The compounds selected can be divided in eight aliphatic acids (ketoglutaric, fumaric, malic, mesaconic, oxalic, pyroglutamic, pyruvic, sorbic) and eleven aromatic acids (4-aminobenzoic, benzoic, *p*-coumaric, ferulic, phthalic, gallic, 4-hydroxybenzoic, homovanillic, protocatechuic, sinapinic and syringic).

Using coelectroosmotic capillary electrophoresis low-molecular-mass organic acids appear at retention times lower than others non-ionized or partially ionized components of beer at pH of work.

To optimize the surfactant concentration and chain length on the resolution, different surfactants such as CTAB, TTAB and HDB were proved. Within the concentration range evaluated, all three surfactants produce flow reversal in a single step [20,21]. The concentration range studied were from 0.03 to 1.9 mM for CTAB, from 0.05 to 5 mM for TTAB and 0.0001–0.02% for HDB (the concentration of the HDB is given in percentage because it does not have a defined molecular weight). This study was carried out varying the modifier concentrations under conditions of constant pH, organic solvent percentage and ionic strength. An increase in surfactant concentration in the bulk has no effect on the extent of adsorption at the surface and the flow magnitude remains practically constant. The best resolution was obtained when HDB was employed at a concentration of 0.001%, which was the one selected.

It has been demonstrated that HDB has some advantages over CTAB as osmotic flow modifier. Firstly, even when used at such low concentrations, HDB produces a positively charged capillary wall coating which is stable for several runs [22] and undesired interactions between hydrophobic analytes and the EOF are significantly reduced.

The effect of pH was studied between 7 and 8.5 observing that when pH increases greater migration times and resolution between the analytes are obtained. The negative charges for the solution interact with the positive charge of double hydrophobic layer formed which is responsible of longer analysis times when the pH increases.

The EOF on a bare capillary decreases with increasing ionic strength. When sodium phosphate concentration is increased the resolution between the electrophoretic peaks also increases. The concentration selected for the rest of the experimental work has been 50 mM.

In order to separate phenolic acids with high separation efficiencies is indispensable to use organic solvents as electrolyte additives as methanol, ethanol, 1-propanol, 2-propanol and acetonitrile [23,24]. A detailed study of presence of organic modifiers was carried out and the best results was obtained with 2-propanol. A study of the percentage of 2-propanol between 15 and 30% indicates that the best resolution was obtained using 25% (see Fig. 1).

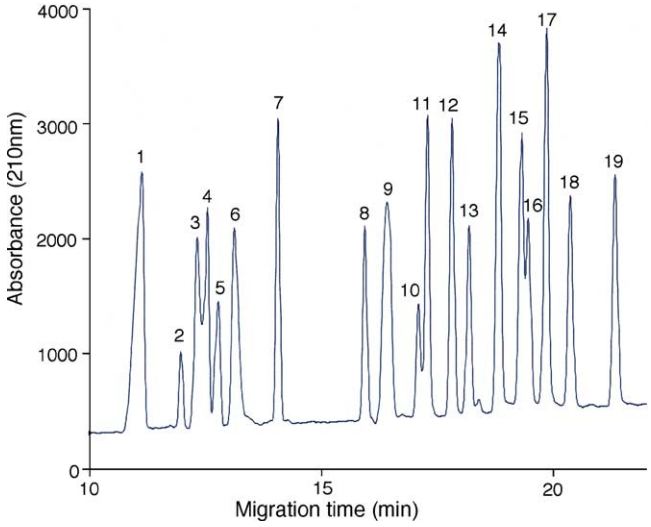

Fig. 1. Separation of aliphatic and aromatic acids by coelectroosmotic CZE. Peaks: (1) oxalic acid (300 mg/l), (2) fumaric acid (10 mg/l), (3) ketoglutaric acid (100 mg/l), (4) mesaconic acid (10 mg/l), (5) malic acid (300 mg/l), (6) pyruvic acid (100 mg/l), (7) phthalic acid (10 mg/l), (8) benzoic acid (10 mg/l), (9) pyroglutamic acid (50 mg/l), (10) sorbic acid (10 mg/l), (11) 4-aminobenzoic acid (10 mg/l), (12) 4-hydroxybenzoic acid (10 mg/l), (13) protocatechuic acid (10 mg/l), (14) gallic acid (10 mg/l), (15) *p*-coumaric acid (10 mg/l), (16) homovanillic acid (10 mg/l), (17) syringic acid (10 mg/l), (18) ferulic acid (10 mg/l), (19) sinapinic acid (10 mg/l). Electrophoretic conditions: 50 mM sodium phosphate (pH 8), 0.001% HDB and 25% 2-propanol, hydrodynamically injection for 7 s; voltage, −15 kV.

3.1. Quantification

Analytical performance characteristics of the proposed method were evaluated. Standard calibration graphs were

Table 1
Analytical characteristics

Analytes	DL (mg/l)	QL (mg/l)	Linear concentration range (mg/l)	R.S.D. (%, mean point)	r^2 (%)
Oxalic acid	0.9	2.9	2.9–600	5.4	97.2
Fumaric acid	0.1	0.4	0.4–25	4.4	98.3
Ketoglutaric acid	0.6	2.1	2.1–250	5.9	95.5
Mesaconic acid	0.1	0.4	0.4–25	4.2	98.3
Malic acid	3.3	11.1	11.1–600	5.5	97.5
Pyruvic acid	0.6	2.0	2.0–250	4.5	98.4
Phthalic acid	0.07	0.2	0.2–25	4.1	98.5
Benzoic acid	0.08	0.3	0.3–25	3.6	98.7
Pyroglutamic acid	0.4	1.3	1.3–187.5	2.4	99.4
Sorbic acid	0.1	0.4	0.4–25	3.5	98.9
4-Aminobenzoic acid	0.04	0.2	0.2–25	3.9	98.5
4-Hydroxybenzoic acid	0.05	0.2	0.2–20	2.6	99.0
Protocatechuic acid	0.05	0.2	0.2–25	4.5	96.9
Gallic acid	0.1	0.4	0.4–24	3.0	98.9
Siringic acid	0.04	0.1	0.1–25	3.5	98.8
Ferulic acid	0.07	0.2	0.2–25	2.5	99.4
Sinapinic acid	0.07	0.2	0.2–25	2.6	99.3

Table 2
Analysis of beer samples

Analytes	Concentration (mg/l)						
	Extra	Classic I	Classic II	Special	Special Black	Light	Non-alcoholic
Oxalic acid (1)	nd	56 ± 2	59 ± 2	nd	nd	nd	nd
Fumaric acid (2)	4.9 ± 0.2	4.4 ± 0.4	4.9 ± 0.3	5.9 ± 0.7	5.4 ± 0.6	nd	nd
Mesaconic acid (4)	nq	4.3 ± 0.2	4.6 ± 0.4	nq	nq	nq	nd
Malic acid (5)	106 ± 12	148 ± 13	156 ± 22	184 ± 24	73 ± 8	128 ± 11	118 ± 11
Pyruvic acid (6)	nq	nq	nq	nq	nq	nq	58 ± 6
Pyroglutamic acid (9)	156 ± 18	124 ± 11	165 ± 12	180 ± 23	152 ± 18	128 ± 12	92 ± 6

nd: non detected, nq: non quantified.

prepared for each analyte. The calibration graphs were linear between different concentrations depending of the analytes studied except for *p*-coumaric and homovanillic acid due its overlap. Wide linear ranges, small standard errors and correlation coefficients indicate very good calibration linearity. The detection (DL) and quantification limits (QL) and precision (expressed in terms of relative standard deviation (R.S.D.) calculated for the mean value of the linear range) were calculated using the method proposed by IUPAC [25]. Three replicates of each analyte at different concentrations were measured in order to set up the calibration. All the features of the proposed method are summarized in Table 1.

3.2. Analysis of organic acids in beer samples

The method could be applicable at any type of matrix containing low-molecular-mass organic acids but the proposed methodology has been proved, in this work, in beer samples.

The beer samples were degassed and filtered through a 0.45 μm filter. The samples were analyzed by standard method adding several amoung of the analytes to a constant volume of sample to verify that no matrix effect exist. The slopes of the calibration graphs and the one obtained by the addition method, were compared using the guidelines proposed by Cuadros Rodríguez and co-workers [26,27].

Among the organic acids studied in all types of beers, the most common ones are: oxalic, fumaric, ketoglutaric, mesaconic, malic, pyruvic and pyroglutamic acid. Fig. 2 shows two electropherograms corresponding to a classic I beer (A) and to a non-alcoholic beer (B). Pyruvic acid cannot be quantified in any type of beer because it overlaps with other unknown compound; only in the non-alcoholic beer pyruvic acid can be quantified as can be seen in Fig. 2.

Table 2 lists the concentrations of low-molecular-mass organic acids found in the different beer samples analyzed. As can be seen from these data, very low error intervals values have been obtained.

Important differences in the concentration patterns of the analytes are related to the fermentation process as well as variations in the brewing procedure. In the case of the non-alcoholic beer, notably low concentrations of all analytes compared to the other beer samples under investigation were found because a dilution in the brewing procedure is usually carried out.

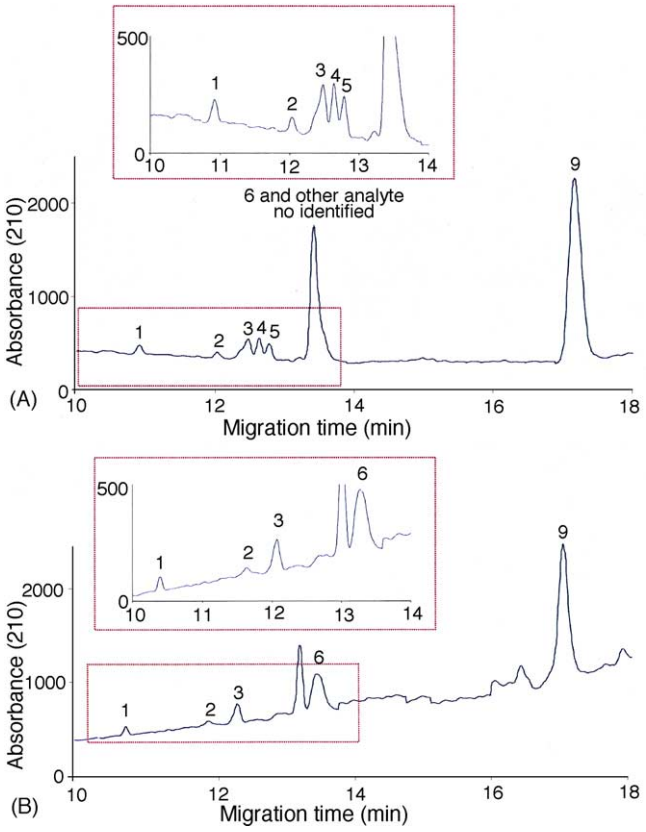

Fig. 2. Electropherograms of (A) classic I beer and (B) non-alcoholic beer. Electrophoretic conditions: 50 mM sodium phosphate (pH 8), 0.001% HDB and 25% 2-propanol, hydrodynamically injection for 7 s; voltage, −15 kV. Peak number's as in Fig. 1.

4. Conclusion

The objetive of this study has been to demonstrate that CZE performed with coelectroosmotic flow might be useful for analyzing low-molecular-mass organic acids present in a very extended alcoholic beverage such as beer. Coelectroosmotic CZE is potentially very useful because of its short analysis time being the times of the total process of 22 min for the analysis of 19 compounds, which make this method suitable for screening in the brewering industry.

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