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0308-8146(95)00060-7

Food Chemistry, Vol. 55, No. 1, pp. 17-21, 1996
Elsevier Science Limited
Printed in Great Britain.
0308-8146/96 $15.00+0.00

RAPID COMMUNICATION

Sensitive determination of ethanol in low-alcohol samples by ion-exclusion chromatography with EC detection using a ruthenium-based inorganic film electrode

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(Received 18 January 1995; accepted 23 March 1995)

Ethanol in real matrices was determined by ion-exclusion chromatography (IEC) with electrochemical detection at a glassy carbon electrode coated with conducting films of mixed-valent ruthenium oxide cross-linked with ruthenium cyanide (mvRuO–RuCN). These films exhibit very high and persistent elecrocatalytic behavior toward oxidation of hydroxyl-containing compounds, and offer attractive features for applications as electrocatalytic amperometric sensor in acidic media. Real samples such as vinegar and low-alcohol beers were simply diluted with mobile phase (1:10), filtered and injected without extensive sample treatment. A polymeric-based hydrogen-form ion-exclusion column was chosen for the chromatographic separations using 25-mM sulphuric acid as the mobile phase. The response was linear for sample solutions containing ethanol up to 0.1 M with a dynamic range of more than three orders of magnitude.

INTRODUCTION

The determination of ethanol in alcoholic beverages is mainly done by gas chromatographic (Harnos *et al.*, 1994) and enzymatic methods (Marko-Varga *et al.*, 1994; Maeder *et al.*, 1994). Gas chromatography (GC) with flame ionization detector may be considered the most common method for alcohol determination in regular wine (Caputi & Mooney, 1983), beer (Cutaia, 1984), and spirits (Ceccon *et al.*, 1993). Although several examples of applications are reported, no specific low-alcohol samples have been investigated. Enzymatic assay methods are recognized to be highly selective and sensitive, but the use of alcohol oxidase (AOD) and alcohol dehydrogenase (ADH) gives rise to group- rather than absolute substrate-specific interaction. Thus, simple aliphatic alcohols have been determined as apparent ethanol content (Huidobro *et al.*, 1994, Chi & Dong, 1994).

In recent years, electrochemical (EC) detection with liquid chromatography (LC) has gained popularity as a sensitive and selective detection technique for electroactive groups. Ethanol and other aliphatic alcohols, which are inherently difficult to detect due to lack of a chromophore or fluorophore group, are also scarcely electroactive compounds. Amperometric anodic detection can occur by catalytic mechanisms on certain transition-metal oxides, e.g. nickel (Casella *et al.*, 1993), and ruthenium (Leech *et al.*, 1991). The detection and quantification of ethanol in liquid-phase with high sensitivity and selectivity are required in many different areas. Much work has been focused on enhancing the detection of aliphatic alcohols at platinum and gold electrodes using dedicated instrumentation with programmed multistep potential waveforms (Johnson & LaCourse, 1990; LaCourse *et al.*, 1991). This approach is needed because these noble metal electrodes undergo a progressive fouling of the electrode surface.

Over the past 10 years, much effort has been devoted to identifying modified electrodes that may exhibit electrocatalytic activity. Of particular interest have been inorganic materials that may give rise to modified electrodes because of their potential utility in a wide range of electroanalytical applications (Cox *et al.*, 1991). Since ruthenium hexacyanometalate complexes are able to form conducting polymeric films on electrode surfaces, some analytical applications have been proposed. Kulesza reported the first investigation on the electrocatalytic oxidation of methanol at a mixed-valent ruthenium oxide–ruthenium cyanide (mvRuO–RuCN) film deposited on glassy carbon electrodes (Kulesza, 1987). Recent studies in this laboratory have illustrated

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the ability of this inorganic film to mediate also the electrochemical oxidation of higher aliphatic alcohols (Cataldi *et al.*, 1995*a*) and aldehydes (Cataldi *et al.*, 1995*c*) in diluted solutions of sulphuric acid.

In the present work a simple, sensitive and precise method is described for determining ethanol in low-alcohol beverages and vinegar without extensive sample clean-up, based on ion-exclusion chromatography (IEC) with electrochemical detection at a mvRuO–RuCN modified electrode. These films exhibit very high and persistent electrocatalytic activity for the oxidation of ethanol and other simple aliphatic alcohols.

EXPERIMENTAL

Reagents and samples

Ethanol absolute, 99.7% (Carlo Erba, Milan, Italy), ruthenium(III) chloride hydrate, and potassium hexacyanoruthenate(II) hydrate (Aldrich Chemical Co.) were used as received. Other chemicals employed were of analytical grade (Aldrich) and were used without further purification. Solutions were prepared in deionized and doubly distilled water. Real samples of low-alcohol beer (Tourtel with ethanol content less than 0.5% v/v, and Buckler with label claim not specified) and vinegar (white wine and apple vinegar, Ponti S.pA., Ghemme, Italy) were purchased from a local store, diluted 10-fold with the mobile phase, filtered through a 0.46-μm syringe filter membrane of cellulose acetate (Sigma Chemical Co.) and injected without further pretreatment.

Electrode preparation

The preparation of mixed-valent ruthenium oxide–ruthenium cyanide film by potential cycling was first described by Cox & Kulesza (1984). Our group very recently reported a more stable and reproducible procedure for electrodeposition when the process is carried out at constant applied potential during a prescribed amount of time (Cataldi *et al.*, 1995*a*). Before each modification, the glassy carbon was wet polished with 0.05-μm α-alumina powder, rinsed with a stream of deionized water and sonicated for a few minutes. The electrodes were then placed in freshly prepared solutions containing 1-mM $K_4Ru(CN)_6$ and $RuCl_3$ in 25-mM H_2SO_4 and the potential held at +1.05 V for 15 min. Straightforward film deposition by controlled potential is carried out and consistently yields results similar to those obtained from films prepared by potential cycling.

Apparatus

The chromatographic system consisted of a Hewlett Packard 1050 pump equipped with an on-line degasser system and a Rheodyne (Berkeley, CA) Model 7125 injector using a 50-μl sample loop. Amperometric detection was performed using a Princeton Applied Research (PAR) Model 400 electrochemical detector and a flow through thin-layer electrochemical cell with a single glassy carbon working electrode (MP 1305), Ag/AgCl reference electrode, and stainless steel auxiliary electrode. The detector time constant was set at 1 s. The output signal was recorded by a X-t Amel Model 868 recorder. All measurements were performed at room temperature, by applying the desired operating potential and allowing the transient current to decay prior to the amperometric monitoring.

Chromatographic conditions

Ion-exclusion chromatographic separations were effected at room temperature (22 ± 2°C) using an Aminex HPX-87-H (BIO-RAD Labs.) cation-exchange resin column, 300 × 7.8 mm i.d. (9-μm particle size) using 25-mM H_2SO_4 as the eluent. In order to maintain a stable electrode response, the mobile phase contained also 5×10^{-6} M $RuCl_3$ and $K_4Ru(CN)_6$ (Cataldi *et al.*, 1995*b*). The flow rate was set at 0.5 ml min^{-1}. In this experimental conditions, no sign of column loss of performance was observed.

The identification of ethanol was made by capacity factor (*k*'), and the quantification was performed by the standard additions method. The Fig. P Ver 6.0c package (Biosoft, Cambridge, UK) was used to obtain the linear regression equations.

RESULTS AND DISCUSSION

Liquid chromatography with electrocatalytic detection

The electroactive inorganic mvRuO–RuCN film electrode, recognized as formed by ruthenium-oxide stabilized with the residual cyano-groups of the hexacyano complex, is able to significantly lower the high overpotential for the oxidation of short chain aliphatic alcohols and aldehydes in acidic media without the presence of alkali ions (Cataldi *et al.*, 1995*a*). On the basis of those previous results, the mvRuO–RuCN modified electrode was extensively investigated as alcohol sensor in liquid solutions in order to explore the possibility of ethanol detection following ion-exclusion chromatography of low-alcohol liquid samples. Figure 1 shows a

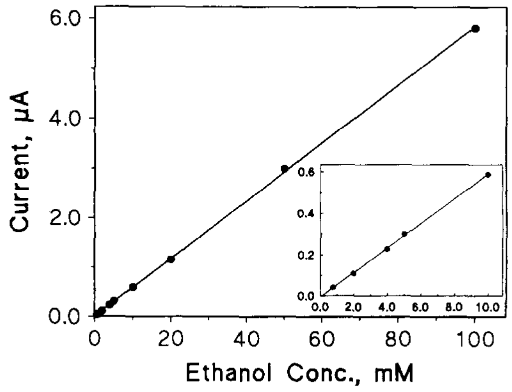

Fig. 1. Calibration curve for ethanol obtained by IEC–EC detection at a mvRuO–RuCN chemically modified electrode. Parameters for the best-fit line are given in the text. The inset shows an enlarged view of the lower five points in the graph.

typical calibration curve obtained for ethanol by LC–EC detection. The best-fit line for this graph has a slope of 0.0584 (± 0.0004) μAmM^{-1}, an intercept of +0.003 (±0.016) μA, and a correlation coefficient of 0.9993 using eight points in the graph. The standard deviation (SD) of slope and intercept was estimated at 95% confidence level. The limit of detection (LOD) for ethanol was determined to be 5×10^{-5} M at a signal-to-noise ratio of three (i.e. $S/N = 3$). The linear range was found to extend up to at least 100 mM, and the dynamic range expressed as the ratio of the upper and the lower limits is 2×10^3. This LOD is comparable with that reported for non-specific ethanol ADH biosensor with electrochemical detection, 0.5 μg ml^{-1} (Maeder *et al.*, 1994) and slightly higher than pulsed amperometric detection at a Pt electrode, 0.2 ppm (LaCourse *et al.*, 1991). However, this modified electrode affords better selectivity (see below) and overall a more extended linear region of the calibration curve.

We previously demonstrated that the mvRuO–RuCN modified electrode can be easily prepared (see Experimental section) in diluted mineral acid solutions and successfully applied in flow injection and chromatographic analysis (Cataldi *et al.*, 1995*a*). Moreover, this inorganic film is stable and can be used for several days in flowing streams provided that the mobile phase/carrier electrolyte is spiked with micromolar concentrations of the ruthenium salts employed in the electrodeposition step (Cataldi *et al.*, 1995*b*, 1995*c*).

Quantitative evaluation of low-ethanol concentrations in real samples

Ethanol plays an important role in several technological fields from food industry and biotechnology to process control. Usually standard gas chromatographic methods are employed for alcohol determination in wine and beer. However, even though GC official methods of analysis (e.g. Official Methods of Analysis, 1990) of the ethanol content in alcoholic beverages are specific, accutare and precise, no examples of applications to low-alcohol samples are reported. In this paper a liquid chromatographic separation with electrochemical detection using a mvRuO–RuCN modified electrode was applied to several matrices to illustrate the analytical utility of the assay. Two different types of commercial vinegar (white wine and apple vinegar) and

Table 1. Ethanol content of vinegar and low-alcohol beers analyzed by IEC–EC detection at a mvRuO–RuCN film electrode

Sample	Estimated concentration[a] $x \pm t\,s_x$ (%, v/v)
Low-alcohol beer (Tourtel)	$0.261 \pm 5 \times 10^{-3}$
Low-alcohol beer (Buckler)	$0.417 \pm 6 \times 10^{-3}$
Apple vinegar (Ponti)	$0.153 \pm 4 \times 10^{-3}$
White wine vinegar (Ponti)	$0.042 \pm 4 \times 10^{-3}$

[a] Concentraction evaluated by the method of standard additions, t is taken at 95% confidence level; s_x represents the standard error of concentration estimated.

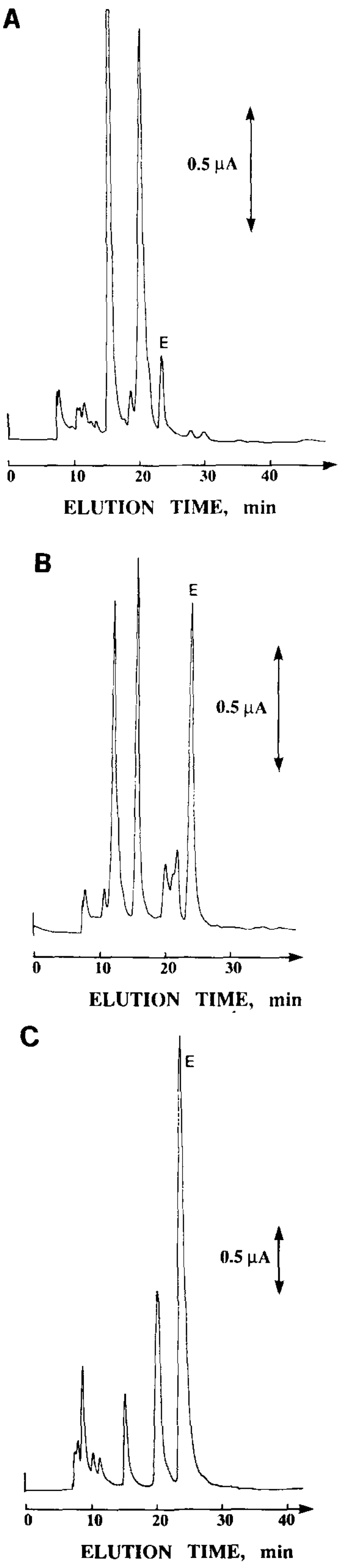

Fig. 2. Liquid chromatographic separation with electrochemical detection of ethanol (E) in commercial white vinegar (A), apple vinegar (B), and low-alcohol beer (C). Column, Aminex HPX-87-H, 300 × 7.8 mm i.d.; isocratic elution with 25-mM H_2SO_4 (room temperature); flow rate, 0.5 ml min^{-1}; loop, 50 μl; thin-layer electrochemical cell with +1.1 V vs Ag/AgCl as applied potential. Samples were diluted 10-fold with mobile phase.

beers were analyzed and the results of the determinations are summarized in Table 1. Figure 2(A–C) shows these applications using a polymeric-based cation-exchange column in the H^+ form, where retention of molecular solutes is likely based on hydrophobic interactions and/or difference in partitioning between the interstitial eluent and the stagnant eluent within the pores of the resin (Fritz, 1991). In all samples examined ethanol was well resolved and detected with excellent sensitivity. In fact, the high selectivity and sensitivity of the mvRuO–RuCN modified electrode contributed to simple sample preparation and simplified chromatograms. Excellent reproducibility was obtained for separations on repetitive injections, and no deterioration of the performance of the modified electrode was apparent. Prior to injection all samples were diluted with the mobile phase 10-fold and filtered. Increasing quantities of a standard solution of ethanol were added to the sample, and chromatographic analysis was carried out using 25-mM H_2SO_4 as the mobile phase. The height of ethanol peak relevant to the original concentration, together with that of spiked samples, was plotted versus the added amount. The unknown content of ethanol was obtained from the regression lines by using the method of standard additions (Miller & Miller, 1988). The correlation coefficients (*r*) of the regression lines were in the range of 0.9992–0.9998. Figure 3 shows two examples of the quantitative determination of ethanol in beers with low-alcohol content. Considering the ease of electrode preparation we typically modified an electrode and employed it in the course of a day. However, the measurements of Fig. 3 were obtained with the same deposit of mvRuO–RuCN on two separate days to demonstrate the reproducibility of sensitivity. The levels of ethanol evaluated (see Table 1), 0.261% (± 2% as relative standard deviation, RSD at 95% confidence level) and 0.417% (± 1.4% RSD) by volume, are consistent with the values provided by the manufacturer (i.e. less than 0.5% ethanol contained in non-alcoholic beers). As Fig. 3 shows, the standard additions method is successfully applied to real samples and allows the determination of the ethanol in a variety of beers.

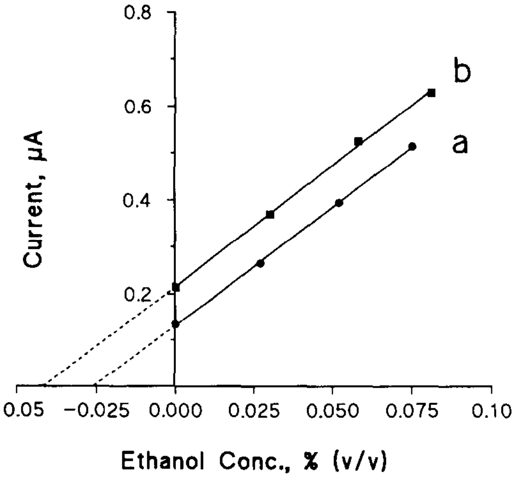

Fig. 3. Calibration curves of ethanol (peak height vs amount spiked in the beers) obtained by sequential standard additions using IEC–EC detection. (a) Tourtel low-alcohol beer; (b) Buckler non-alcoholic beer. Experimental conditions were the same as those described in Fig. 2.

CONCLUSION

A very sensitive and accurate method for ethanol determination with minimal sample handling is proposed, which combine the advantages of an ion-exclusion chromatographic separation with the selectivity and sensitivity of electrochemical detection at a ruthenium-based modified electrode. This method has proved to be very effective, reliable and well suited not only for the analysis of relatively high concentrated alcoholic drinks such as whisky, wine, beer, etc., but also can successfully determine ethanol in low-alcohol wines and beers, and low-alcohol liquid samples.

ACKNOWLEDGEMENTS

The financial assistance of the Italian National Research Council (C.N.R., Rome) and Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T., Rome) are gratefully acknowledged.

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