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Notes

Determination of Acetaldehyde in Fuel Ethanol by High-Performance Liquid Chromatography with Electrochemical Detection

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The cyclic voltammetric behavior of acetaldehyde and the derivatized product with 2,4-dinitrophenylhydrazine (DNPHi) has been studied at a glassy carbon electrode. This study was used to optimize the best experimental conditions for its determination by high-performance liquid chromatographic (HPLC) separation coupled with electrochemical detection. The acetaldehyde-2,4-dinitrophenylhydrazone (ADNPH) was eluted and separated by a reversed-phase column, C_{18}, under isocratic conditions with the mobile phase containing a binary mixture of methanol/$LiCl_{(aq)}$ at a concentration of 1.0×10^{-3} M (80:20 v/v) and a flow rate of 1.0 mL min^{-1}. The optimum condition for the electrochemical detection of ADNPH was +1.0 V *vs*. Ag/AgCl as a reference electrode. The proposed method was simple, rapid (analysis time 7 min) and sensitive (detection limit 3.80 μg L^{-1}) at a signal-to-noise ratio of 3:1. It was also highly selective and reproducible [standard deviation 8.2% ± 0.36 (n = 5)]. The analytical curve of ADNPH was linear over the range of 3 – 300 mg L^{-1} per injection (20 μL), and the analytical recovery was > 99%.

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Introduction

In Brazil, the use of ethanol as an automotive fuel or an oxygenated gasoline additive has dramatically increased since 1975 as a consequence of the National Alcohol Program launched as a strategic way to become more independent of oil imports. Currently, the annual production of Brazilian fuel ethanol is around 14 billion m^3/year, which classifies it as one of the world's biggest producers.[1]

The presence of inorganic and organic contaminants in fuel ethanol is rigorously controlled by a Federal Regulatory Agency (Agência Nacional do Petróleo) according to limits for each impurity content. Although, these contaminants are considered to be a secondary products, they have primordial importance when modern engines are used, which are developed with high technology, and therefore require fuel with a high degree of purity. Since the use of fuel ethanol is very restricted, the availability of sensible, rapid and selective analytical methods for the quantification of organic contaminants is scarce.

Acetaldehyde is the most significant impurity seen in fuel ethanol at different levels of concentrations, which depends on the manufacturing procedure, storage and other factors. Although its importance is well-known, there are only a few articles in the literature dealing with the proposed alternative methods for its identification and quantification, which is very important for quality control purposes, such as to know the chemical composition in detail.

Most HPLC methods concerning to acetaldehyde are based on a reaction with 2,4-dinitrophenylhydrazine (DNPHi) to improve the sensitivity of the analysis of carbonyl compounds. This purpose was very well established by Kissinger *et al*. in the detection of these compounds with electrochemistry detection.[2]

Nevertheless, a HPLC method for acetaldehyde in fuel ethanol using electrochemical detection has not been described. We report on the development of a simple methodology for the quantification of acetaldehyde in fuel alcohol based on HPLC with electrochemical detection.

Experimental

Reagents

All of the chemicals used were of ACS reagent grade or better. Acetaldehyde was from Merck (Darmstadt, Germany). All solvents used, such as methanol, ethanol and acetonitril, were of HPLC grade from Mallinckrodt (Xalostoc, Mexico). Demineralized water was obtained from a Milli-Q Water System (Millipore, CA, USA). The 2,4-dinitrophenylhydrazine (DNPHi) from Merck (Darmstadt, Germany) was purified by three successive recrystallizations from methanol. H_3PO_4 was from Aldrich Chemical Co. (Milwaukee, USA). LiCl and LiOH, obtained from Aldrich Chemical Co. (Milwaukee, USA), were used as supporting electrolytes in demineralized water.

Derivatization of acetaldehyde-2,4-dinitrophenylhydrazone (ADNPH)

ADNPH used as a standard was prepared by the well-known reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPHi), obtained as described.[3] DNPHi (0.4 g; *ca*. 2 mmol) was dissolved in concentrated phosphoric acid (2 mL) and demineralized water (3 mL). To this solution, the standard acetaldehyde (1 g), dissolved in ethanol (15 mL), was added. The reaction product was isolated *via* filtration and purified (twice) by recrystallization from absolute ethanol.

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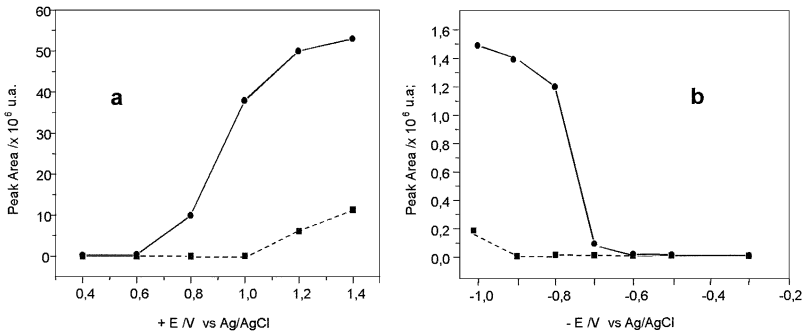

Fig. 1 Hydrodynamic voltammograms of the standard ADNPH 50 mg L^{-1} solution at a glassy carbon electrode to (a) the oxidation and (b) the reduction mode. Mobile phase (···) containing methanol/$LiCl_{(aq)}$ 1.0×10^{-3} mol L^{-1} (80:20 v/v).

Standard solutions were prepared by dissolving weighed amounts (1 g L^{-1}) of pure ADNPH in acetonitrile. Portions of these solutions were then diluted with suitable ethanol–water (95:5 v/v), to minimize the matrix effects; also, standard solutions for calibration purposes were prepared in the concentration range of 3 – 300 mg L^{-1}.

Sample derivatization

A 0.4% solution of DNPHi was prepared by dissolving DNPHi (0.4 g; *ca*. 2 mmol) in acetonitrile (100 mL). In a volumetric flask, 0.900 mL of a DNPHi solution, 4.0 mL of the sample (without previous concentration) and 50 µL of H_3PO_4 1.0 mol L^{-1} were introduced, consecutively. The resulting solution was stirred at room temperature for 20 min. Sample derivatives were filtered through a 0.45 µm filter from Millipore (Millipore, CA, USA) and injected into the HPLC system.

Chromatographic conditions

A ProStar Varian HPLC apparatus was used under isocratic conditions with a mobile phase consisting of methanol/$LiCl_{(aq)}$ 1.0×10^{-3} M (80:20 v/v). A Rheodyne Model 7725 injection valve with a 20 µL sample loop was used. A reversed-phase column, Shimadzu Shim-pack C_{18} (150 × 6.0 mm i.d., 5 µm), was applied for all measurements with a guard column (Shimadzu Shim-pack C_{18}).

The electrochemical detector (Varian ProStar 370), based on the wall-jet principle, consisted of a glassy carbon working electrode (area = 0.0707 cm^2), a silver-silver chloride reference electrode and a glassy carbon counter electrode was also used. Prior to use, mobile phase solvents were filtered through a 0.45 µm filter (Millipore) and degassed with high-purity nitrogen. The electrochemical detector potential was set at +1.0 V and the flow rate was 1.0 mL min^{-1}.

Voltammetric conditions

Voltammetric measurements were performed using a potentiostat/galvanostat (EG&G Princeton Applied Research Corp. (PARC), Model 173). A voltammetric cell containing three electrodes was used: a glassy carbon electrode (Methrom, area = 0.0314 cm^2) as working electrode, a platinum wire as counter electrode and a saturated calomel electrode as reference (Methrom). The working electrode was polished between experiments using a 0.05 µm alumina slurry, and rinsed with demineralized water.

The voltammetric parameters were an equilibration time of 15 s and a scan rate of 50 mV s^{-1}. Solutions of LiCl (1 mol L^{-1}) and LiOH (1 mol L^{-1}) as well as methanol/$LiCl_{(aq)}$ (1.0 mol L^{-1}; 50:50 v/v) and acetonitrile/$LiCl_{(aq)}$ (1.0 mol L^{-1}; 50:50 v/v) were used as supporting electrolytes. All of the solutions were purged with high-purity nitrogen for 10 min prior to recording voltammograms, and a continuous stream of nitrogen was passed over the solutions during measurements.

Quantitative analysis

A quantitative conversion of acetaldehyde in fuel ethanol to a derivative form (ADNPH) was achived by using a large excess of DNPHi.[4-6] The standard curve for ADNPH was obtained by linear regression, plotting the peak area *vs*. concentration. The correlation coefficient was very close to unity.

Analysis of fuel ethanol samples

Different commercially available fuel ethanol samples were collected around Araraquara, São Paulo, Brazil. These samples were stored in pyrex glass flasks at a temperature controled to 20°C during all measurements. The acetaldehyde contents in these samples were derivatized and the analyses were carried out on a HPLC system.

Results and Discussion

HPLC separation of standard ADNPH

No adsorption problem at the electrode surface was found while acetaldehyde measurements for producing oxidation and reduction hydrodinamic voltammograms were undertaken. To obtain analytical measurements for the working electrode on the HPLC system, it was necessary to carefully polish its surface initialy using 0.05 µm alumina slurry, and before each different potencial it was necessary to realize a clean electrochemistry program set at +1.0 and –1.0 V during 20 s.

The effect of the potential on the hydrodynamic voltammogram recorded for standard ADNPH 50 mg L^{-1} at different working potentials using a mobile phase containing methanol/$LiCl_{(aq)}$ (1.0×10^{-3} mol L^{-1}; 80:20 v/v), is shown in Fig. 1. However, the oxidation curve obtained for ADNPH under the experimental conditions described above, is shown in Fig. 1a. Although a small signal was observed at +0.8 V, the maximum values of the peak current were observed at a potential higher than +1.0 V. The optimal working potential for the oxidation of ADNPH was chosen at around +1.0 V. At higher potentials than +1.2 V, an increase in the background current was found, and therefore increases in the noise were also observed. The correspondent hydrodinamic voltammogram to reduction (Fig. 1b) indicated that there was no significant signal at values lower than –0.7 V, but the maximum signal was obtained at a potential higher than –0.9 V, where

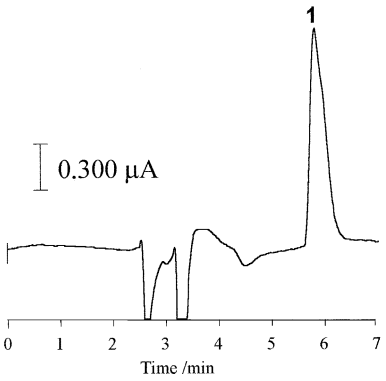

Fig. 2 HPLC chromatogram of the standard ADNPH 50 mg L^{-1} solution on a Shimadzu C_{18} column (150 × 6.0 mm i.d.; 5 μm) with a flow rate of 1.0 mL min^{-1}. Mobile phase, methanol-$LiCl_{(aq)}$ 1.0×10^{-3} M (80:20 v/v). Electrochemical detection was at a glassy carbon electrode set at +1 V. Identification of peak, 1, ADNPH.

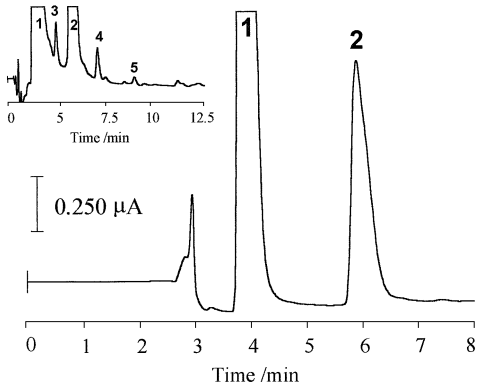

Fig. 3 HPLC chromatogram of the an ADNPH fuel ethanol sample on a Shimadzu C_{18} column (150 × 6.0 mm i.d.; 5 μm) with a flow rate of 1.0 mL min^{-1}. Mobile phase, methanol-$LiCl_{(aq)}$ 1.0×10^{-3} M (80:20 v/v). Electrochemical detection was at a glassy carbon electrode set at +1 V. Identification of peaks: 1, DNPHi; 2, ADNPH; 3, 5-hydroxymethylfurfural; 4, furfural; 5, butylaldehyde.

both nitro groups were probably being reduced.

To select the optimum detection potential, it is desirable to maximize the current response while minimizing the background noise. In the case of the ADNPH, both potentials of –0.8 V or +1.0 V could be used from hydrodynamic voltammograms. Taking this into consideration, chromatograms for standard ADNPH (50 mg L^{-1}) were recorded using a reversed-phase column (Shimadzu Shim-pack C_{18}), accomplished under isocratic conditions using mobile phase methanol/$LiCl_{(aq)}$ (1.0×10^{-3} mol L^{-1}; 80:20 v/v) at a flow rate of 1.0 mL min^{-1}. The chromatograms obtained under a reductive potential presented a high contribution of background current due the interference of electroactive dissolved oxygen that was not removed even under a long time of desaeration conditions. Thus, optimization *via* the oxidation potential was investigated.

Figure 2 shows a typical chromatographic separation for standard ADNPH with electrochemical detection. As can be seen, good chromatographic separations of ADNPH were obtained under the optimized experimental conditions, and the elution of the examined compound was completed in a time of less than 7 min during a chromatographic run. Peak identification was based on the retention time (5.80 min), and was confirmed by spiking authentic standard solutions of ADNPH.

The analytical curve for electrochemical detection, based on the peak area *vs*. concentration, was preferred instead of the peak height for ADNPH because of a possible poisoning of the electrode surface, which would be prejudicial to the quantitative purposes.[7] The analytical curve for ADNPH was constructed by plotting the peak area against the concentration, and a linear relationship was obtained from 3 to 300 mg L^{-1}, the parameters of which were as follows: area = $1.52 \times 10^5 + 0.10 \times 10^5$ [ADNPH] with $r^2 = 0.9998$. The detection limit (L.O.D.) determined at the lowest injected concentration, taken as a signal-to-noise ratio equal to 3:1, was 3.80 μg L^{-1}.

Determination of acetaldehyde in fuel ethanol samples

Using the best experimental conditions, defined previously, several fuel ethanol samples from different sources were treated with DNPHi, and aliquots were analyzed by HPLC with an electrochemical detector operating at +1.0 V, where the best separation was obtained. The inset in Fig. 3 shows a characteristic chromatographic separation obtained for a typical commercial fuel ethanol sample. The presence of a relatively high concentration of DNPHi did not produce any visible effects on the signals of the ADNPH product generated after the reaction, which were sufficiently separated from each other. In addition to the peak corresponding to the ADNPH, some extra peaks were present, and further work identified almost all of them. These additional peaks are attributed to the presence of other carbonyl contaminants. In addition, the method was tested for several fuel ethanol samples; the relevant quantitative results are listed in Table 1. In general, the acetaldehyde content in fuel ethanol samples is significantly high, and the values point to a high concentration of acetaldehyde in the fuel, changing from concentration of 83 to 343 mg L^{-1}. These findings reflect the difficulties associated with a complex matrix.

Table 1 Concentration of ADNPH determined in fuel ethanol samples and analytical recoveries added to its samples[a]

Sample	Concentration present/ mg L^{-1}	Concentration added/ mg L^{-1}	Concentration found (± SD)	Recovery, % (± SD)
S_1	83.0	21.0	103 ± 0.02	99 ± 2
S_2	139	35.0	176 ± 0.05	101 ± 2
S_3	201	50.0	255 ± 0.34	102 ± 3

a. $n = 5$, injections were performed in duplicate.

The recoveries were evaluated for ADNPH using fuel ethanol samples spiked with a standard derivative solution of ADNPH at a level of around 25% of the measured content and performing five assays after each addition (Table 1). The concentration of ADNPH was calculated using the area of the peaks. It was calculated by a linear regression approach using the method of standard addition.

The reproducibility of the method was ascertained by carring out five assays on the same sample over two days in duplicate. The value of the standard deviation was low (8.2% ± 0.36) and the coefficient of variation was 3.2%. Nevertheless, the proposed method has shown sufficient selectivity and sensitivity to its determination.

Conclusions

An optimized high-performance liquid chromatography with an electrochemical detection method for the determination of acetaldehyde in fuel ethanol samples was successfuly developed. In particular, the electrochemical detector has shown the advantage of higher sensitivity, and also offers selectivity, which is very useful when analyzing real samples, because it reduces any matrix effects, and consequently improves the quantification of acetaldehyde. Therefore, our findings led to a rapid, reliable and simple method for its quantification and identification in fuel ethanol.

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