Interest of Pulsed Electrochemical Detection for the Analysis of Flavor-Active Alcohols Separated by Liquid Chromatography

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Electrochemical detection of short-chain aliphatic alcohols separated by liquid chromatography demonstrates close sensitivities at gold and platinum working electrodes. Addition of acetonitrile in the eluent decreases the sensitivity of detection (peak area) at the gold electrode but improves the apparent sensitivity (peak height) by a consequent decrease of retention time. At the platinum electrode, the detection was completely inhibited by traces of acetonitrile. Gold was therefore chosen to investigate the amperometric detection of flavor-active alcohols, and particulary of terpenols, separated by reversed-phase chromatography using an acetonitrile–water (1:1) eluent. Primary alcohols were detected at the ppb level (20–300 ppb) and secondary and tertiary alcohols at the ppm level (0.1–6 ppm). Primary and secondary alcohols can be quantified with good repeatability and sensitivity in a wide range of concentrations. Unsaturation in the alcohols drastically improves the limits of detection due to a better adsorption at the electrode surface. Tertiary alcohols can be easily detected at two different optimum potentials, but are more difficult to quantify accurately.

INTRODUCTION

Flavor chemists mainly use high-resolution gas chromatography (HRGC) for separating volatile constituents (Lawrence and Shu, 1993). However, this technique is readily substituted for HPLC when a large number of samples has to be analyzed (Maarse, 1991) or when relatively large amounts of material are required for further identification by HRGC–MS (Schreier, 1988) or NMR. HPLC can also be preferable to HRGC when experiments in aqueous media need a regular control; a typical example is the control of the production of volatile metabolites during fermentation.

Nevertheless, the use of HPLC is limited because of the difficult detection of most volatiles by UV, rarely including sensitive chromophores, as is the case for alcohols (Scott, 1964; ϵ_{max} = 200 for methanol at λ = 177 nm).

Recent publications have demonstrated that short-chain alcohols could be detected with interesting sensitivities and limits of detection using on-line amperometric detection at a gold or a platinum electrode (Hughes et al., 1981; LaCourse et al., 1991; Johnson and LaCourse, 1990, 1992; Mead et al., 1989).

The aim of this paper is to investigate the possibility of using such a detector for the analysis of flavor active C_6–C_{10} alcohols. After a comparison of the relative performances of gold and platinum as working electrodes for the detection of short chain alcohols, a gold electrode was chosen for the analysis of these flavor-active alcohols. The parameters of detection and separation were determined to optimize their analysis. The effect on sensitivity of acetonitrile, as a necessary cosolvent to elute these compounds in reversed-phase chromatography, was also studied.

MATERIALS AND METHODS

HPLC. The eluent (a mixture of water and acetonitrile) was degassed with helium using an eluent degas module from Dionex. Separations were realized on columns packed with C18-bound silica (Lichrosorb 10 μm, 250 × 4.6 mm from Interchim and lichrospher 5 μm, 250 × 4 mm from Merck) connected to an advanced gradient pump from Dionex (Sunnyvale, CA) via a Rheodyne 9126 injector equipped with a 25-μL peek (poly(ether ether ketone)) loop. Detection was made with a pulsed electrochemical detector (PED, Dionex). The postcolumn addition of an electrolyte (NaOH (0.3 M) for detection at gold electrode and $HClO_4$ (0.1 M) at platinum) was necessary to ensure the conductivity of the solution. It was made possible by a reagent delivery module (RDM, Dionex) at 1 mL/min. The RDM module was connected after the analytical column by a mixing tee. Nitrogen was used to pressurize the RDM to avoid formation of sodium hydrogen carbonate when NaOH was used. A 124-cm 500-μL beaded reaction coil was placed after the mixing tee to facilitate the mixing of the reagent and eluent streams with minimal band broadening.

The flavor-active alcohols were eluted with a mixture of water and acetonitrile (1:1) and detected at a gold working electrode. Because of the respective flows coming from the column and from the alkali container, PED detection was consequently made in a water/acetonitrile mixture (3:1) containing NaOH (0.15 M). Short aliphatic alcohols were eluted with pure water and detected on either gold (with postcolumn addition of NaOH (0.3 M)) or platinum (with postcolumn addition of $HClO_4$ (0.1 M)) electrodes.

Solvents and Reagents. Standard flavor-active alcohols were obtained from Sigma or Aldrich and were used after their purity was checked by HRGC. Methanol and 2-propanol (HPLC grade) were purchased from Carlo Erba and ethanol (HPLC grade) and ethylene glycol from SDS (France). Sodium hydroxide was a "Baker analyzed" reagent from J. T. Baker (Holland). Perchloric acid was a RP Normapur Prolabo (France) reagent.

Amperometric Detector Cell. A thin-layer cell was used with a silver/silver chloride reference electrode and the stainless body of the cell as counter electrode. The working electrode was a round gold electrode (1.4- or 3-mm diameter) included in a block of inert Teflon material. The thickness of the cell was set up to 76 μm with the largest electrode and to 50 μm with the other, thus corresponding, respectively, to 3.5- and 1.25-μL internal volumes. The platinum electrode size was identical to the largest gold electrode. The cell was installed in a poly(styrene) box to avoid base-line drift with temperature. To avoid the accumulation of adsorbed species at the electrode surface, a multistep potential waveform was applied to restore the native reactivity: after the detection process, the electrode was cleaned by the application of a very positive potential step, called E_{ox}, to oxidatively remove the adsorbed species and then was

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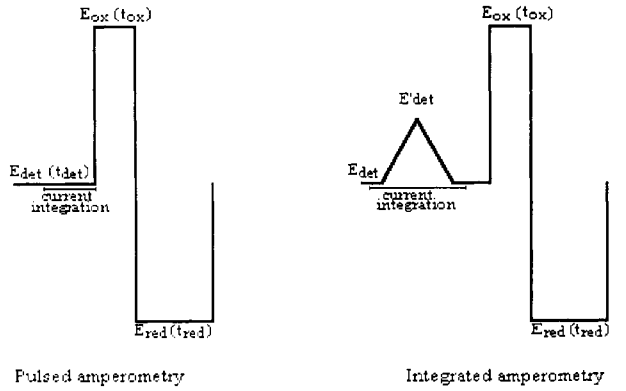

Figure 1. Potential–time waveforms: E_{det}, E'_{det}, anodic detection; E_{ox}, oxidative cleaning; E_{red}, cathodic reactivation. The integration period is 200 ms for pulsed amperometry and 400 ms for integrated amperometry.

reactivated by a large cathodic potential step, called E_{red}, to reduce the oxide formed.

Two amperometric modes of detection are available using this kind of multistep potential waveform (Figure 1). The first mode of detection, or pulsed amperometric detection, is appropriate to the detection of molecules which have their oxidation potential in the region of oxide-free surface. The detection potential (E_{det}) was determined in relation to the oxidation potential and in order to optimize the signal to noise ratio. To detect species oxidized on the gold oxide wave, it was necessary to sweep the voltage between the oxidation potential and a potential located in an oxide free region. The integration was made between these two potentials so that the anodic current due to oxide formation during the positive scan tends to be compensated for by the cathodic current produced when the oxide is reduced during the negative scan. This technique is called integrated amperometry.

Determination of the Working Potential. For the gold electrode, the best working potential was estimated by two different methods: first, by cyclic voltammetry using an external system with a gold rotating disk electrode (Au RDE: d = 2 mm; 600 rpm) connected to a PRT potentiostat and a UAP4 unit (both from Tacussel) with a calomel reference electrode and a platinum counter electrode, and second, with repetitive injections in HPLC–PED by measuring the effects of changing the working potential on peak height. For the platinum working electrode, only the second process was used. The potential sequence applied to the gold working electrode was the following: E_{det} from 0 to 500 ms; E_{ox} = 0.7 V from 510 to 630 ms; E_{red} = −0.65 V from 640 to 900 ms. The oxidation current was integrated from 300 to 500 ms at E_{det}. For the platinum working electrode, the potential sequence was as follows: E_{det} = 0.22 V from 0 to 300 ms; E_{ox} = 1.4 V from 310 to 430 ms; E_{red} = −0.4 V from 440 to 800 ms. The current was integrated from 280 to 300 ms.

RESULTS AND DISCUSSION

This study mainly concerns unsaturated C_6–C_{10} alcohols compared to a saturated alcohol, 1-hexanol.

Choice of the Detection Electrode. For the HPLC–PED analysis of aliphatic alcohols, a platinum working electrode is recommended (Rocklin, 1989; Mead et al., 1989). LaCourse et al. (1991) demonstrated that short-chain alcohols could be detected in better conditions using a platinum rather than a gold electrode. *n*-Propanol produced a much higher oxidation wave in cyclic voltammetry in acidic media at the platinum electrode than in basic media at the gold electrode (Johnson and LaCourse, 1992). Conversely, carbohydrates are recommended to be analyzed at gold (Neuburger and Johnson, 1987; Rocklin and Pohl, 1983; Edwards and Haak, 1983), and Beden et al. (1987) showed that ethylene glycol, glycerol, and 2-butanol were better detected at gold than at platinum electrodes by cyclic voltammetry in alkaline medium. Thus, it was necessary to compare the two types of electrodes in order to choose the best working electrode for detecting flavor-active alcohols.

Table 1. Detection of Aliphatic Alcohols at both Platinum and Gold Electrodes

	t_R (min)	limit of detection (ppm (pmol))	linearity (ppm)	r^2	av noise (nA)
Platinum Electrode without CH_3CN					
ethanol	6.3	0.33 (180)	45	0.9950	6
ethylene glycol	3.7	0.2 (80)	30	0.999 86	6
1-propanol	14.5	0.6 (250)	500	0.998 24	6
2-propanol	12.8	12 (5160)	>1000	0.999 98	6

	t_R (min)	limit of detection (ppm (pmol))	linearity	r^2	av noise (nA)
Gold Electrode without CH_3CN					
ethanol	6.3	1.5 (815)	>400	0.999 14	0.6
ethylene glycol	3.7	0.06 (24)	10	0.999 97	0.6
1-propanol	14.5	4.5 (1875)	>1000	0.999 99	0.6
2-propanol	12.8	2.6 (1080)	>1000	0.999 94	0.6

	% CH_3CN	t_R (min)	limit of detection (ppm (pmol))	linearity (ppm)	r^2
Gold Electrode with CH_3CN					
ethanol	15	4.2	1 (540)	>400	0.999 96
1-propanol	13	5	0.7 (292)	>1000	0.998 61
2-propanol	13	4.5	1.27 (530)	>1000	0.999 79

The first experiment to compare the sensitivity of detection of these two noble metal electrodes toward short-chain alcohols was accomplished using the same chromatographic conditions except for postcolumn effluent which was NaOH for gold and $HClO_4$ for platinum. The eluent was pure water. The waveform for pulsed electrochemical detection at platinum was that used by LaCourse et al. (1991) except for E_{det} which was optimized from a hydrodynamic voltammogram of ethanol showing a maximum of sensitivity at +0.22 V/Ag/AgCl. The same process was used to choose the waveform at gold from the hydrodynamic voltammogram of ethanol showing an optimum working potential at +0.14 V/Ag/AgCl. The four short chain alcohols chosen were tested using these waveforms. The integration time was maintained at 200 ms at gold and chosen at 20 ms for platinum because the observed average noise at platinum was 10-fold higher than at gold.

The comparison (Table 1) showed that both gold and platinum allow the detection of short-chain alcohols with close sensitivity. The limits of detection were lower for ethanol and 1-propanol at platinum and lower for ethylene glycol and 2-propanol at gold. The possible range for quantification was better with gold as indicated by the linearity of the detection. Moreover, the experiment reported in Table 1 demonstrated that the detection of three alcohols at gold is easier when acetonitrile is added to the eluent. This conclusion is different from that given by LaCourse et al. (1991) due to the fact that these authors compared the detection with gold and platinum using different chromatographic conditions, i.e., different types of columns and different eluent compositions. The same authors attribute the weak sensitivity they observed at gold to a strong adsorption of acetonitrile at the surface of the electrode.

For detecting higher homologues with more interesting odors (Meilgaard, 1975) the comparison between the two types of electrodes is not possible using water as a solvent or using ionic chromatography since the elution time is rapidly too large, i.e., 55 min for pentanol on an AS-1 column using $HClO_4$ (0.05 M) at 0.8 mL/min as eluent

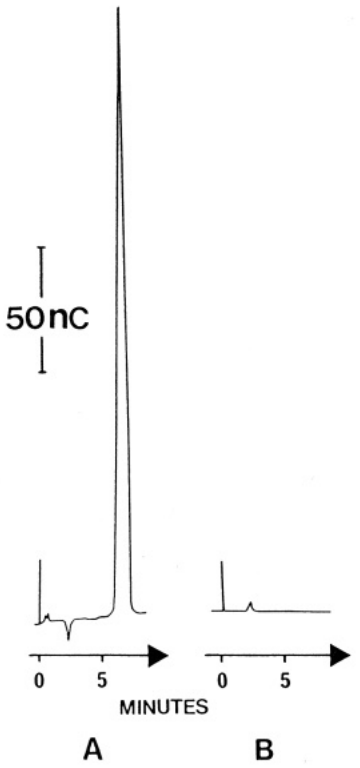

Figure 2. Detection of ethanol at the platinum electrode with and without acetonitrile in the eluent: concentration, 400 ppm; Column, C18 10-μm Interchim; E_{det} = 0.22 V (0–0.3 s), E_{ox} = 1.4 V (0.31–0.43 s), E_{red} = –0.4 V (0.44–0.86 s), integration, 0.18–0.20 s. A: 100% H_2O eluent. B: H_2O/CH_3CN (95.5:0.5) eluent.

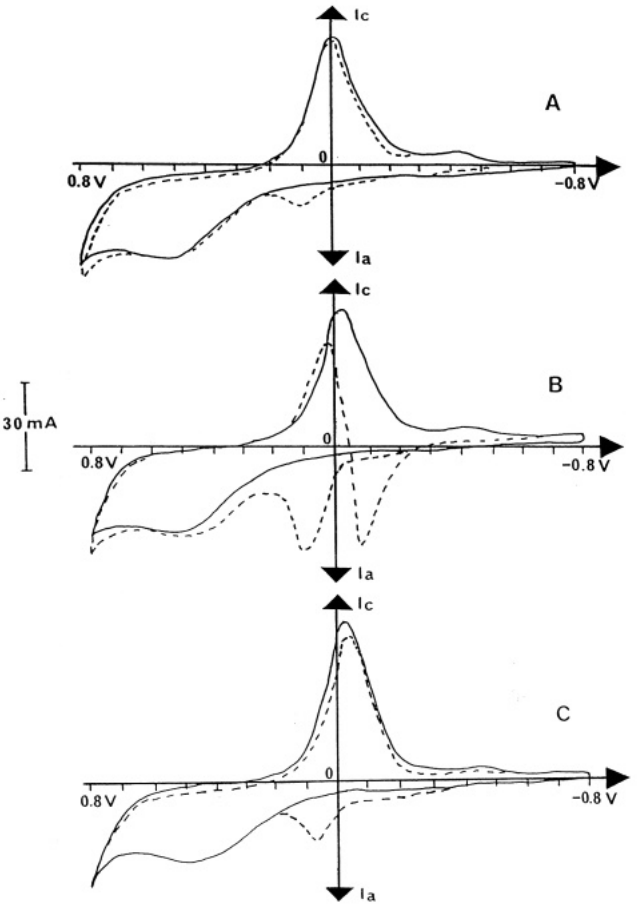

Figure 3. Voltammetric response (i-E) of flavor-active alcohols at a gold rotating disk electrode. Conditions: 0.16 M NaOH, 600 rpm rotation speed, 200 mV s^{-1} scan rate, calomel reference. Solutions: (···) degassed electrolyte without alcohol; (—) degassed electrolyte with alcohol; A, hexanol (10^{-3} M); B, *cis*-3-hexenol 10^{-3} M; C, menthol (10^{-3} M).

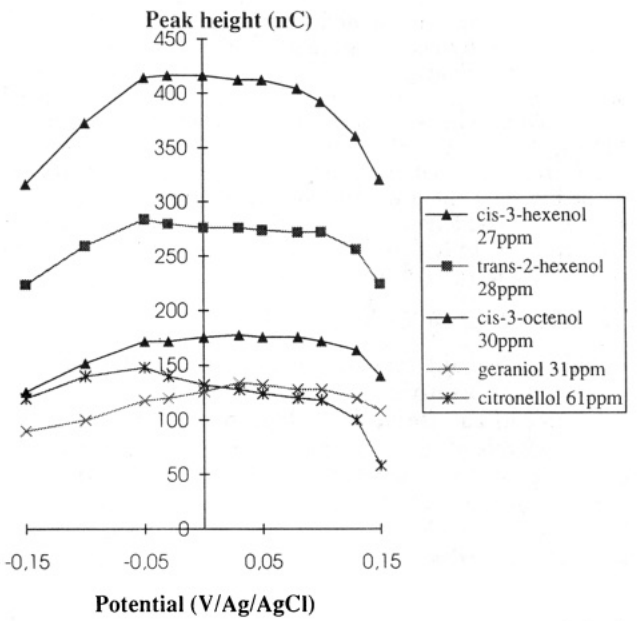

Figure 4. Hydrodynamic voltammograms of primary alcohols at gold using pulsed amperometric detection. Conditions: C18, 10-μm Lichrosorb (Interchim) column, CH_3CN/H_2O (1:1) eluent at 1 mL/min, NaOH 0.3 M postcolumn reagent at 1mL/min. E_{det} (varying), t_{det} = 500 ms; E_{ox} = 0.7 V, t_{ox} = 120 ms; E_{red} = –0.65 V, t_{red} = 360 ms. Integration: 300–500 ms. Reference: Ag/AgCl.

(LaCourse et al., 1991). So, since PED requires the addition of an electrolyte, the only choice was to use reversed-phase chromatography with an organic modifier. Acetonitrile was chosen as cosolvent because it is a common reversed-phase HPLC eluent and because it is compatible with electrochemical detection at gold working electrode although its adsorption is not negligible. On the other hand, acetonitrile is not compatible with electrochemical detection at platinum. Figure 2 clearly demonstrates that 0.5% acetonitrile in the eluent completely inhibits the detection of ethanol at platinum in acidic media (0.05 M $HClO_4$). The drastic passivation of the platinum electrode observed confirms the strong interference of acetonitrile in the adsorption mechanism of alcohols at platinum in acidic media. Besides, it is well known that, under basic conditions, the oxidation of simple alcohols at the platinum electrode occurs simultaneously with the formation of surface oxide and in the region of dissolved O_2 reduction (LaCourse et al., 1991).

Determination of Detection Potentials. Figure 3 shows typical voltammograms of two primary alcohols (hexanol and *cis*-3-hexenol) and one secondary alcohol (menthol) obtained at the gold RDE electrode. The best conditions for the detection of primary alcohols can be determined easily from the wave corresponding to their oxidation, i.e., near +0.15 V/Ag/AgCl. A second wave, on the negative scan, can also be noticed from Figure 3, particularly for *cis*-3-hexenol, indicating that the recovery of surface reactivity, when the gold oxide film is cathodically removed from the electrode surface, makes the oxidation of these electroactive alcohols at 0 V/Ag/AgCl again possible.

Conversely, the voltammograms of the tertiary alcohols studied do not show significant differences when the alcohol is added to the solution (not shown). They only show a decrease in gold oxidation and in gold oxide reduction waves, indicating an adsorption of the alcohols at the electrode surface. Figures 4 and 5 show the

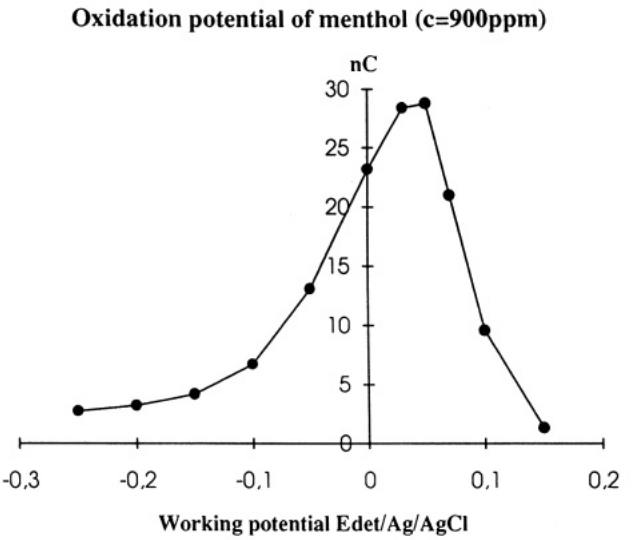

Figure 5. Hydrodynamic voltammograms of menthol 900 ppm and linalool 100 ppm at the gold electrode using pulsed amperometric detection. Conditions: C18, 5-m Lichrospher (Interchim) column, CH_3CN/H_2O (1:1) eluent at 1 mL/min, NaOH 0.3 M 1 mL/min postcolumn addition. E_{det} (varying), t_{det} = 500 ms; E_{ox} = 0.7 V, t_{ox} = 120 ms; E_{red} = −0.65 V, t_{red} = 360 ms. Integration: 300–500 ms. Reference: Ag/AgCl.

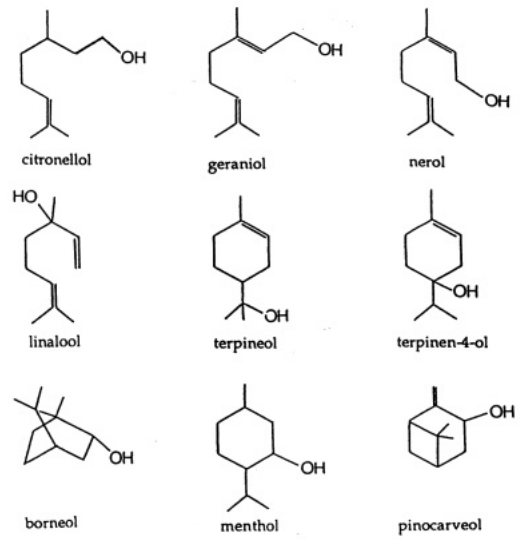

Figure 6. Developed formulas of C_{10} alcohols.

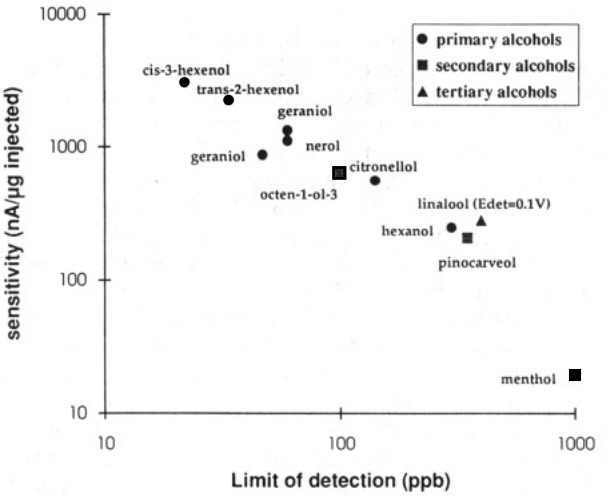

Figure 7. Relation between sensitivity and detectability at optimum conditions for primary, secondary, and tertiary alcohols.

hydrodynamic voltammograms obtained with the PED using a stepwise increase of the working potential. The curves thus obtained confirm that all the primary alcohols studied could be detected in the best conditions in the range −0.05/+0.05 V/Ag/AgCl. Figure 5 shows that tertiary alcohols can be detected with the PED, in opposition to the previous statements. This method allows us to determine the optimum potential for the oxidation of secondary alcohols at +0.05/+0.1 V and at +0.3/+0.4 V/Ag/AgCl for tertiary alcohols. Another anodic peak was observed in the range −0.25/−0.3 V/Ag/AgCl for the unsaturated tertiary alcohols studied (linalool, α-terpineol, terpinen-4-ol). This second peak gives rise to a higher anodic current than obtained at the positive oxidation potential, but at this voltage the noise was greater, and the base line was observed to drift toward more anodic values. This potential corresponds to the lower potential of detection available with this apparatus (LaCourse and Johnson, 1991).

Limit of Detection and Sensitivity. Table 2 itemizes the names of the different alcohols studied, with the optimum conditions found for their detection, and the values determined for sensitivity, limit of detection, linearity range, and repeatability of measures. All these values were determined in the same chromatographic conditions, i.e., a C18 column, an eluent acetonitrile/H_2O (1:1) at 1 mL/min, and a postcolumn addition of NaOH (0.3 M) at 1 mL/min. The chemical structures of the compounds studied are given in Figure 6.

The lower amounts detectable for the different alcohols studied were between 0.02 and 6.1 ppm at the optimum voltages determined (Table 2). This limit is lower for primary alcohols, and unsaturation seems to favor lower limits of detection. For example, the minimum amount detectable is 300 ppb for 1-hexanol and 30 ppb for the corresponding unsaturated *trans*-2- and *cis*-3-hexenols.

Secondary alcohols were detected from concentrations varying between 100 and 1000 ppb, and as for primary alcohols, saturated molecules were detected less easily (menthol) than unsaturated ones. This phenomenon was even more obvious for tertiary alcohols as saturated alcohols such as 2,2-dimethylpropanol or 3,3-dimethylbutanol (not shown) were undetectable. The minimum amount detected for tertiary alcohols varied between 250 and 6100 ppb.

As seen in Figure 7 the sensitivity is well correlated with the limit of detection for the values obtained between 0 and 0.1 V, meaning that the limits of detection and sensitivity vary accordingly. The results obtained at −0.2 V and by integrated amperometry with tertiary alcohols

Table 2. Quantitative Parameters of Primary, Secondary, and Tertiary Alcohols at a Gold Electrode in 0.15 M NaOH

compd	LOD (ppm)	E_1 (V/Ag/AgCl)	R^2	linearity (ppm)	sensibility (nA/μg injected)	repeatability % rsd (ppm; n)
cis-3-hexenol	0.022	0	0.999 998 5	350	3048	1.1 (7.06; 3)
trans-2-hexenol	0.034	0	0.997 63	250	2218	0.37 (8.52; 3)
cis-3-octenol	0.06	0	0.999 996 4	>100	1320	
nerol	0.06	0	0.999 992 6	350	1100	
geraniol	0.047	0	0.999 636	>100	867	
citronellol	0.142	0	0.9969	250	560	
hexanol	0.3	0.1	0.999 62	>150	250	1.7 (16.67; 5)
octen-1-ol-3	0.1	0.1	0.999 78	200	640	8.9 (0.32; 4)
pinocarveol	0.35	0.1	0.999 982	200	210	
menthol	1	0.05	0.9998	>250	19.5	5 (18.7; 3)
linalool	0.25	integrated	0.9933		563	1.3 (12.64; 3)
	0.4	0.1	0.999 92	150	285	26.9 (31.3; 3)
	4.5	−0.2	0.996 61		220	12.1 (134; 3)
α-terpineol	3.2	integrated			210	
terpinen-4-ol	6.1	integrated	0.9999		59	
	0.4	−0.2	0.999 84	120	1050	

were not reported on this graph because of the low repeatability of the measures and the instability of the base line.

Linearity. Different concentrations of each alcohol were successively injected in order to estimate the precision of their quantification using the PED (Table 2). The correlation coefficient for each alcohol was calculated to be $R^2 > 0.998$. The error in repeatability of repetitive injections was estimated to be lower than 2% for primary alcohols and lower than 10% for secondary alcohols. The range of linearity of the detector was also estimated. For primary and secondary alcohols it was estimated to be from 500 (hexanol) to 15 000 (*cis*-3-hexenol) times the detection limits. For tertiary alcohols, the range of linearity was more difficult to estimate. A very low linearity was frequently observed when integrated amperometry or pulsed amperometry at the negative oxidation potential were used. These poor results have to be linked to the noisy and unstable base line observed with these potential waveforms. Consequently, the quantification of tertiary unsaturated alcohols is difficult to realize with the PED. Primary and secondary alcohols are easily to quantify from low (20–300 ppb) to high (100–350 ppm) concentrations.

Influence of Acetonitrile Percentage on the Detection of Alcohols at the Gold Electrode. The area and the height of the peak detected for geraniol using increasing proportions of acetonitrile in the eluent were measured and reported in Figure 8 as a function of the inverse of retention time. These results show that an increase of the proportion of acetonitrile from 16.5 to 31% in the detector (corresponding to 33 to 62% in the chromatographic eluent) resulted in a 13% decrease of the peak area, but conversely in a 400% increase of the peak height because of the consequent decrease of the retention time of the peak. When the percentage of acetonitrile was increased beyond 62% in the chromatographic eluent, the peak height began to decrease from its maximum. The correlation of peak height versus peak area/retention time ratio was observed to be linear ($R^2 > 0.95$) during the evolution of eluent composition (data not shown). The same decrease of the actual detection sensitivity and increase of the apparent sensitivity was observed for 2-propanol in Figure 8 when the acetonitrile percentage in the detector was increased up to 6.5% (13% in the eluent). Beyond this maximum the apparent sensitivity began to decrease. When changing from 0 to 13% acetonitrile in the eluent, the peak height was increased by a factor 172% and the peak area was decreased by a factor 13%. As for geraniol, the correlation between peak height and peak area/retention time ratio is linear, with a correlation factor $R^2 > 0.988$.

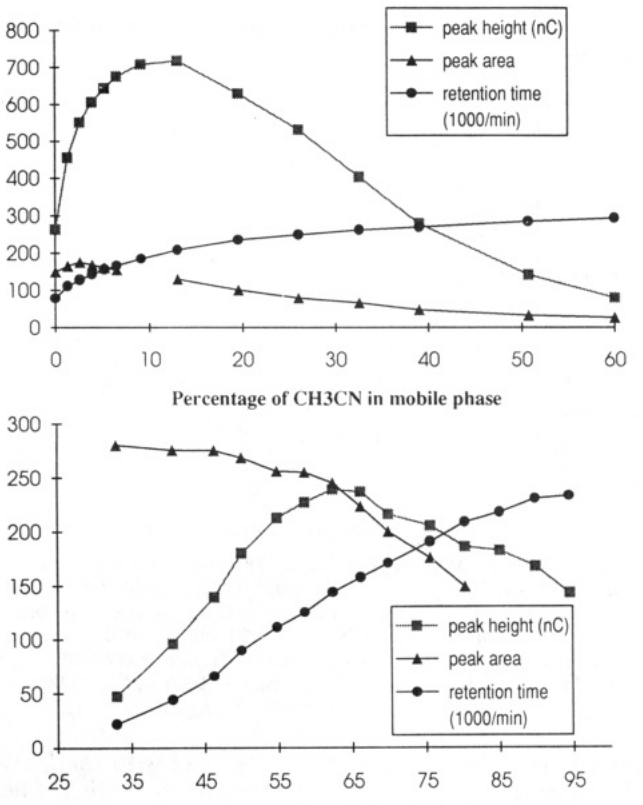

Figure 8. Influence of acetonitrile percentage in eluent on peak height, peak area, and (retention time)$^{-1}$ for 2-propanol (1000 ppm) (top) and geraniol (50 ppm) (bottom). Conditions: gold working electrode. Column: C18, 10-μm Lichrosorb (Interchim). CH_3CN/H_2O (1:1) 1 mL/min. Postcolumn reagent: NaOH (0.3 M) 1 mL/min. Reference: Ag/AgCl. E_{det} = 0.14 V for 2-propanol and −0.05 V for geraniol. Pulsed waveform: E_{det} (0–500 ms), E_{ox} = 0.7 V (510–630 ms), E_{red} = −0.65 V (640–900 ms). Sampling: 300–500 ms.

CONCLUSION

The determination of the working electrode is better made using PED than cyclic voltammetry (CV) because the conditions involved in this determination were identical to the detection conditions (LaCourse and Johnson, 1993). The differences observed between the oxidation potentials with the PED and the CV were probably due, first, to the

hydrodynamic (PED) and the static (CV) systems and, second, to the difference between the multistep potential waveform of PED and linear potential sweep of CV.

The positive voltage determined for the detection of primary alcohols is in agreement with the values found by LaCourse et al. (1991) (+0.1 V/Ag/AgCl). We also observed a logical increase of the potential necessary to oxidize the primary (−0.05/+0.05 V), the secondary (0.05/0.1 V), and the tertiary (0.2/0.3 V) alcohols in agreement with their chemical properties: acidity decreasing from primary to tertiary alcohols.

The negative potentials found for the detection of the tertiary alcohols studied is more difficult to explain. As far as we know, detection of tertiary alcohols at an electrochemical detector and at potentials below −0.2 V/Ag/AgCl at the gold electrode have never been published. Some experiments are currently being performed to clarify this point, but it seems obvious by now that this detection could not be explained by a simple direct oxidation of the hydroxyl group, as aliphatic saturated alcohols were not detected under the same conditions. However, this detection is important since it corresponds generally to higher current intensities, despite a higher background noise and consequently a worse repeatability. The positive oxidation potential of tertiary terpenic alcohols is also difficult to explain since tertiary saturated alcohols were not detected in this region. Since linalool is detected with more sensitivity than α-terpineol and terpinen-4-ol, we can suspect an oxidation of the molecule involving the double bonds, or the production of an anodic current via an isomerization, or a rearrangement of the molecule adsorbed at the electrode surface.

The limits of detection determined for alcohols were fairly low for primary alcohols. Secondary and tertiary alcohols were also detected in reasonable concentrations, although with higher detection limit concentrations. This difference was higher than normal for linalool because the detection was realized at a potential lower than the optimum (0.1 V instead of 0.2–0.3 V). This choice was made because the oxidation of gold begins at approximately 0.15 V, thus increasing considerably the noise of the detector and decreasing the reactive surface of the electrode. An elegant solution provided by the pulsed electrochemical detector was to scan the potential up and then down from the negative to the positive optimum and then back to the negative one while measuring the current intensity all over this pulse. Unfortunately, due to the noise, the base line drift, and the low repeatability observed in this mode, good linearity was not obtainable. So, the quantification of tertiary terpenic alcohols by this technique remains difficult. Conversely, the detector response was proved to be linear for primary and secondary alcohols from the limit of detection up to high concentrations. Moreover, the accuracy of the measurements was similar to that expected in HPLC analysis using other detectors, confirming the interest of using a pulsed electrochemical detector for quantifying alcohols.

It is clear that in HPLC–PED, the two conditions necessary to detect a molecule are its electroactivity and its capacity to adsorb at electrode surface. The occurrence of one or several double bonds in the molecule makes detection easier. The influence of the position and multiplicity of these double bonds is more difficult to ascertain. It is possible that the *Z* configuration of the unsaturation may favor the detection (see *cis*-3- and *trans*-2-hexenol) by a better adsorption at the surface of electrode. For the same reason, an increase in the number of double bonds appears to favor the detection (see geraniol and citronellol). This increase in sensitivity is, however, negligible compared to the improvement due to the "addition" of one single double bond to a saturated alcohol.

Our results showed that increasing the percentage of acetonitrile, as it is generally necessary in HPLC separations of natural aromatic extracts, resulted mainly in a decrease of the alcohols peak areas, confirming the partial passivation of gold electrode by acetonitrile (LaCourse and Johnson, 1990). However, because increasing the percentage of acetonitrile also decreases the retention times of alcohols, the apparent sensitivity estimated as the peak height increases regularly until a maximum corresponding to 62% acetonitrile for geraniol and to 13% acetonitrile for 2-propanol. This effect is more obvious for long-chain alcohols because their retention times are more affected by the addition of a same percentage of acetonitrile than short-chain alcohols.

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