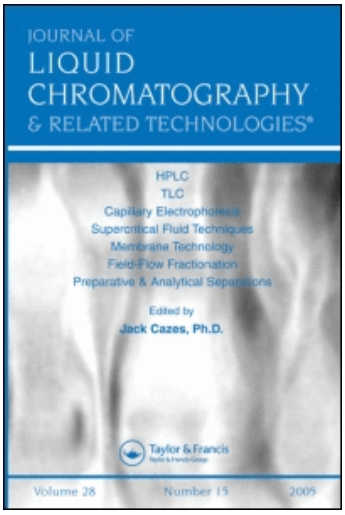

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Electrochemical Detection of 2,6-Diisopropylphenol (Propofol) in Reversed Phase HPLC at High pH

Diego E. Pissinis[a]; Juan M. Marioli[a]

[a] Departamento de Química, Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina

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Diego E. Pissinis and Juan M. Marioli
Departamento de Química, Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina

Abstract: The electrochemical response of propofol (2,6-diisopropylphenol) at glassy carbon electrodes was studied by cyclic voltammetric experiments under different experimental conditions. The voltammetric peak current and potential for the oxidation of propofol were analyzed at different scan rates, pHs, propofol concentrations, and organic modifier amounts. The results obtained helped to optimize the setup parameters for the amperometric detection of propofol in HPLC. The use of mobile phases at high pH significantly lowered the detection potential and chromatographic capacity factors. The effect of the organic modifier amount on the chromatographic capacity factor of propofol was also evaluated.

Keywords: Propofol, Cyclic voltammetry, Amperometric detection, HPLC, Polymeric columns

INTRODUCTION

Propofol (2,6-diisopropylphenol) is a short acting intravenous administered anesthetic largely used for induction and maintenance of anesthesia in human and veterinary medical practice.[1] Propofol has two important advantages as an anesthetic: a short induction period and a relatively short and predictable action.[2] Also, its rapid metabolism and large distribution clearance relative to distribution volume result in a unique disposition that is well suited

J.M.M. is a member of CIC-CONICET.
Address correspondence to Juan M. Marioli, Departamento de Química, Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, ruta 36, km 601, X5804 BYA, Río Cuarto, Argentina. E-mail: jmarioli@exa.unrc.edu.ar

for both the induction and maintenance of anesthesia.[3] Given the widespread use of propofol, precise analytical methods are required to monitor its concentration in body fluids.

A variety of methods have been published describing the quantification of propofol. These procedures have generally employed reversed phase high performance liquid chromatography (RP-HPLC) on silica based C_{18} columns with either UV,[4] fluorimetric,[5–7] or mass spectrometric detection techniques.[8] However, they often have low sensitivity, the equipment employed is expensive, and/or require highly trained personnel for their use.

Electrochemical detection in flowing solutions offers a selective and sensitive tool for the determination of a wide variety of compounds in body fluids and tissues.[9] For example, norepinephrine, other catecholamines and their major metabolites were analyzed by capillary electrophoresis with electrochemical detection in the amperometric mode. The analytical system showed good response sensitivity, low mass limits of detection, and excellent response reproducibility.[10] Moreover, electrochemical detection has been previously employed for the quantification of propofol after chromatographic separations.[11,12] The electrochemical detector consisted of porous graphite working as flow-through electrodes in coulometric mode, which offers good sensitivity and selectivity. The chromatographic separations were achieved with phenyl[11] and C_{18}[12] reversed phase columns and acidic mobile phases containing over 60% (v/v) organic modifiers to facilitate the elution of the solute.

Propofol is a weakly acidic compound ($pK_a = 11.65$),[13] which can be ionized at high pH. Retention of acidic, as well as basic, solutes in reversed phase chromatographic columns is strongly influenced by the pH of the mobile phase,[14,15] ionized compounds eluting earlier than unionized ones. On the other hand, the oxidation potential of phenol and phenol derived molecules is strongly influenced by their ionization state. The potential for the oxidation of phenol like molecules shifts to lower values when they lose the phenolic proton.[16] This dropping of the oxidation potential means that a lower potential will be necessary at the working electrode of the amperometric detector, and a smaller baseline noise may be obtained. Smaller baseline noises imply that enhanced signal-to-noise ratios, i.e., better sensitivity, may be observed. Thus, it seems reasonable to think that the manipulation of the ionization state of propofol can be conveniently used to control the chromatographic capacity factor, and to enhance the sensitivity for its amperometric detection. Consequently, in this work we studied the effect of pH on: a) the electrochemical response of propofol at glassy carbon electrodes, b) the current response of propofol to an electrochemical detector working in the amperometric mode, and c) the retention behavior of propofol on reversed phase columns. We also studied the effect of the amount of an organic modifier on the current response of propofol in a mobile phase of pH 13.8, and the current response of the amperometric cell against the concentration of propofol.

EXPERIMENTAL

Equipment

A conventional three compartment cell was used for the voltammetric experiments. The working electrode was a 3 mm diameter glassy carbon disk (GC) inserted into a teflon tube. The electrode was polished thoroughly with alumina and cleaned in an ultrasonic bath before each measurement. The counter electrode was a stainless steel foil. All potentials were corrected for *iR* drop by positive feedback techniques. The cyclic voltammetric experiments were carried out with an Eco Chemie Autolab PGSTAT 30 potentiostat. The signals were processed using the Autolab General Purpose Electrochemical Software (GPES).

The chromatographic system consisted of a Gilson 307 solvent delivery module (Gilson, France), a Rheodyne 7125 injection valve with a 20 μL loop (Rheodyne, USA), and a Hamilton PRP-1 column of 150 × 4.1 mm packed with 5 μm particles (Hamilton, USA) coupled with a guard column with the same stationary phase. A home made potentiostat was used as an amperometric detector. The electrochemical signal was fed to a PC compatible computer equipped with Peak Simple data processing software (SRI, USA). The electrochemical flow cell for the liquid chromatographic experiments consisted of a Bioanalytical Systems model MF-1000 glassy carbon working electrode (Bioanalytical Systems, USA), a stainless steel auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. The dead volume of the thin layer flow cell was approximately 5 μL.

pH measurements were made with an Orion model 720A bench top pH/ISE meter. The pH meter was calibrated at pH 7.00 and pH 10.00 before its use.

Reagents

Propofol was purchased from Aldrich (USA). Phosphoric acid, acetonitrile (HPLC grade), and sodium hydroxide were purchased from Merck (Argentina). Solutions were prepared in HPLC grade water. HPLC quality water was obtained from a Labconco (USA) WaterPro Mobile reverse osmosis system. All experiments were performed at 298 K.

RESULTS AND DISCUSSION

Cyclic Voltammetry

The electrochemical behavior of propofol (5.0×10^{-4} M) in a 90:10 (v/v) mixture of buffer-ACN at glassy carbon electrodes (GC) and different

pHs was studied by cyclic voltammetry. The potential at the working electrode was scanned from −0.2 V to 0.9 V and back to −0.2 V. A typical cyclic voltammogram of propofol at pH = 3.0 is shown in Figure 1. A well defined oxidative wave can be observed at approximately 0.68 V on the positive going potential scan (peak I) with no reversal peak on the negative going potential scan, even at the highest potential scan speed used in this study. This behavior suggests that the oxidation of propofol is an irreversible process where a chemical reaction follows the initial electro transfer.

A reductive wave can be observed at approximately 0.27 V on the negative going potential scan (Figure 1, peak II). Another well defined oxidative wave is observed at approximately 0.47 V on the second scan toward positive potential values (Figure 1, peak III). This peak can be considered complementary of peak II. Taking into account the proposed mechanism of electrochemical oxidation of phenol like molecules,[17] it may be anticipated that the electrochemical oxidation of propofol produces a radical, which reacts to produce another molecule (a dimeric product in principle) that is further oxidized at potentials around that of peak I. Then, the reduction of this product may be detected as the potential of the working electrode is scanned toward negative values, and its oxidation observed on successive oxidative cycles. This reaction mechanism, known as ECE (electron transfer–chemical reaction–electron transfer) is frequent in organic electrochemistry,[18] particularly of phenol like molecules.[19] Moreover, these steps are repeated on repetitive cycling of the electrode potential, producing the progressive fouling of the electrode surface and a decrease of the electrochemical response, as can be observed in Figure 1, dotted line trace (third voltammetric scan).

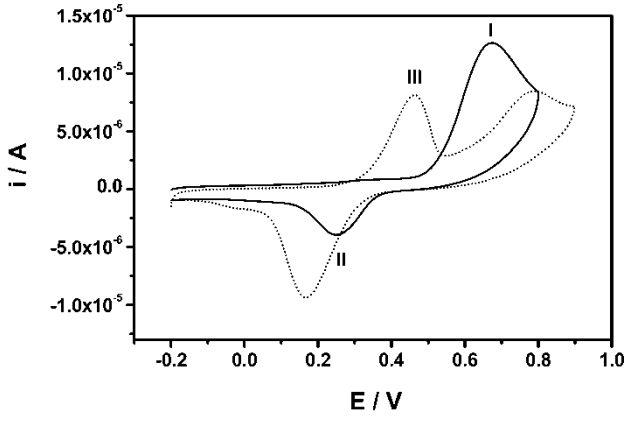

Figure 1. Cyclic voltammetric response of 5.0×10^{-4} M propofol in 90:10 (v/v) buffer-ACN pH 3. Scan rate 0.040 V/s. (—) first, and (. . .) third voltammetric cycles.

The dependence of the peak current of wave I with the scan rate was studied at different proportions of buffer-ACN. The results of the linear regression of i_p vs $v^{1/2}$ (peak current versus the square root of the scan rate) are summarized in Table 1. There is a linear relationship between i_p and $v^{1/2}$ (Figure 2A and Table 1). Thus, it may be proposed that the electron transfer between propofol and the GC electrode is reversible. Also, the shape of the plot of the current function:

$$\Psi = \frac{i_p}{v^{1/2} \cdot C^*},$$

where C^* is the molar concentration of the solute against $v^{1/2}$, exhibits a shape indicative of chemical reactions coupled to the electron transfer reaction (Figure 2B).[20]

A decrease of the peak current of wave I can be observed as the amount of ACN in the electrolyte solution is increased. These results are implicit in the smaller slopes observed in the study of the dependence of i_p vs $v^{1/2}$ (Table 1). They mean that higher sensitivities may be obtained with a decrease in the amount of ACN in the mobile phase. Thus, from an analytical point of view, it is important to keep the amount of ACN in the mobile phase as low as possible.

The electrochemical response of propofol at GC electrodes in 90:10 (v/v) buffer-ACN electrolytes at pH = 13.8 (not shown) is similar to that at pH = 3.0, but the peak potentials of the equivalent waves are shifted toward negative potential values. The pK_a value for propofol in 90:10 (v/v) buffer-ACN mixtures is approximately 11.93.[13] Thus, propofol is mainly present as an anion at pH 13.8, the phenolate derived ion. It is known that phenolate ions are oxidized at lower electrode potentials as compared to the neutral species.[19] As a consequence, it may be proposed that propofol oxidation is observed at approximately 0.10 V, while those related to the reduction/oxidation of the product of the coupled chemical reaction are detected at approximately −0.28 V and −0.20 V, respectively. It is worth noting the lower oxidation potential of propofol at pH = 13.8, as compared to that obtained at pH = 3.0. This smaller oxidation

Table 1. Parameters obtained from the linear regression ($y = B * x$) of the voltammetric peak current versus the square root of the potential scan rate at different amounts of organic modifier. Propofol concentration: 5.0×10^{-4} M

	Organic modifier amount (v/v)		
	90:10	70:30	50:50
B	$4{,}31 \times 10^{-5}$	$2{,}83 \times 10^{-5}$	$2{,}06 \times 10^{-5}$
r	0,9946	0,9956	0,9941

B: slope; r: correlation coefficient.

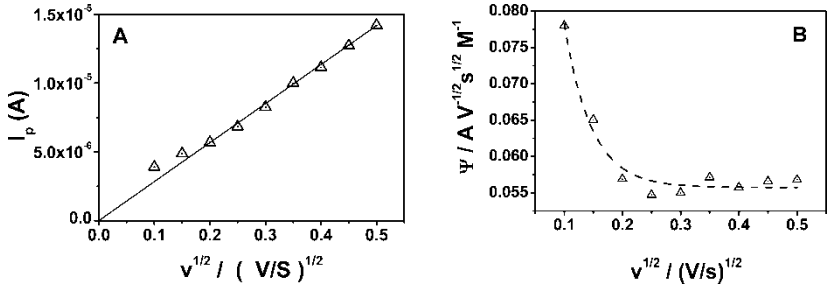

Figure 2. Dependence of (A) voltammetric peak current, and (B) current function (see text) with the square root of the potential scan rate. Propofol concentration: 5.0×10^{-4} M. Solvent: 70:30 (v/v) buffer-ACN mixture, pH 3.0.

potential may be conveniently used for the constant potential amperometric detection of this molecule in HPLC.

Liquid Chromatography

Liquid chromatographic experiments were performed to evaluate various analytical parameters, such as working electrode potential, capacity factor, and linear range of the working electrode response. Thus, we analyzed the hydrodynamic voltammograms (HDVs) of propofol at different pHs, the effect of the amount of organic modifier in the mobile phase on the capacity factor of propofol, and propofol calibration curves.

The hydrodynamic voltammograms of propofol in a mobile phase of 60:40 (v/v) buffer-ACN, at different pHs, are shown in Figure 3. Trace A in Figure 3 shows the response obtained in the study performed at pH = 8.2. The initial working potential was −0.10 V. After obtaining a stable, drift free baseline, five separate injections of propofol were performed and the chromatographic peak current (I_p) was computed. The working potential was then increased by increments of 0.10 V, and the analysis was repeated. In the region between −0.10 V and 0.40 V there is essentially no evidence for propofol oxidation. At potentials higher than 0.50 V the peak current increases and then plateaus at values above ca. 1.10 V. The "half wave potential" (the potential at half the plateau current) is ca. 0.80 V. The capacity factor (defined as: $k' = (t_r - t_0)/t_0$, where t_r is the solute retention time and t_0 is the transit time of an unretained solute) of propofol under the above mentioned chromatographic conditions was around 20. Trace B in Figure 3 shows the response obtained in a similar study performed at pH = 13.8. The electrochemical oxidation of propofol is evident at potentials above 0.20 V, and the peak current plateaus at potentials greater than 0.70 V. The half wave potential at pH = 13.8 is ca. 0.33 V, smaller than the half wave potential at pH = 8.2. The capacity factor of

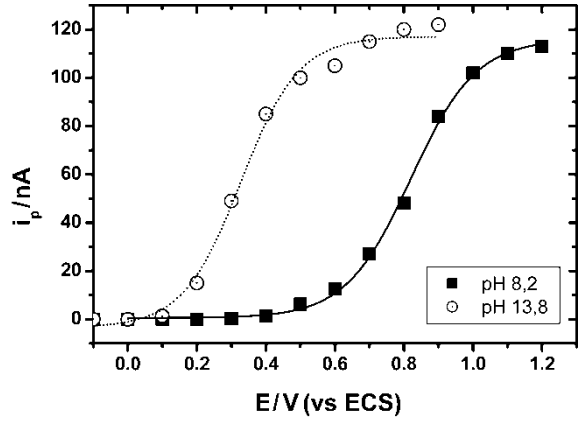

Figure 3. Hydrodynamic voltammograms of 1.0×10^{-4} M propofol in 90:10 (v/v) buffer-ACN mixtures at different pHs. Flow rate = 0.50 mL/min.

propofol at pH = 13.8 is ca. $k' = 2$. In conclusion, these analyses show that a smaller capacity factor may be obtained (faster analysis time per sample) and a smaller set potential at the working electrode of the electrochemical detector may be set if an alkaline mobile phase is used.

A chromatographic calibration curve was made for propofol to test the linearity of the electrode response with changes in the amount of injected propofol. The mobile phase consisted of a 60:40 (v/v) buffer-ACN mixture at pH = 13.8. A wide linear concentration was obtained, with an excellent correlation coefficient for the logarithmic analysis ($\log(I_p)$ vs. $\log(C^*)$). The results of the linear regression ($y = A + B * x$) are: $A = 4.740$, $B = 0.820$, and $r = 0.999$. Moreover, the signal-to-noise ratio obtained with the lowest propofol concentration used for this study was ca. S/N = 8, which means that even lower propofol concentrations might be analyzed. Thus, a propofol concentration of approximately 3×10^{-8} M (equivalent to 5 ng/mL or 600 fmol of injected propofol) may be taken as the limit of detection (LOD) of the method at a S/N ratio of 3.

The dependence of the solute's capacity factor with the volume fraction (Φ) of the organic modifier in the mobile phase was also studied at pH = 13.8. The degree of reduction of a solute's retention is related to Φ by the equation:

$$\log(k') = \log(k')_w - S * \Phi$$

where $(k')_w$ is the capacity factor of the solute with a completely aqueous mobile phase and S is the slope coefficient, which defines a solvent strength parameter. Good correlation coefficients were obtained ($r \geq 0.99$) for the logarithmic plot. A value of ca. 0.034 was obtained for the slope coefficient

from the logarithmic plot. This value seems reasonable given the size of the solute under study.[21]

CONCLUSIONS

Cyclic voltammetric experiments of propofol showed that this molecule is oxidized through a complex mechanism, probably involving coupled chemical reactions. The study of the pH effect on the voltammetric parameters (peak current and potential for propofol oxidation) revealed that propofol can be oxidized at low electrode potentials when high pH solutions are used. This observation was conveniently used for the amperometric detection of propofol in HPLC, using highly alkaline mobile phases and reversed phase polymeric columns. The calibration curve for the amperometric detection of propofol at pH 13.8 showed a wide linear range, with a limit of detection of approximately 3×10^{-8} M at a signal-to-noise ratio of 3. The ionization of propofol at high pH also produced a decrease in its chromatographic capacity factor, which may be translated into lower amounts of organic modifier required for elution of the analyte. Also, higher oxidative currents can be obtained by using lower amounts of organic modifier in the mobile phases.

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