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Voltammetric study and determination of buprenorphine in pharmaceuticals

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Abstract

The oxidation of buprenorphine on a carbon paste electrode has been studied using voltammetric techniques under both semi-infinite linear diffusion and hydrodynamic conditions. By applying a simple electrode pretreatment a good reproducibility of the current signal is obtained (R.S.D. = 0.85%, $n = 6$ for a 1.0×10^{-5} M buprenorphine concentration). The limit of detection was found to be 2.0×10^{-7} M. The voltammetric method developed for the determination of buprenorphine in pharmaceutical preparations was examined for its applicability to liquid and solid preparations.

Keywords: Buprenorphine; Pharmaceuticals; Voltammetry; Carbon paste electrodes

1. Introduction

Buprenorphine, [5a,7a (s)]-17-(cyclopropylmethyl)-α-methyl-6,14-ethenemorphinan-7-methanol (Fig. 1), is a synthetic opioid analgesic. Analgesic doses in man are less than half a milligram and blood concentrations are in the nanogram range. Its effectiveness as a postoperative analgesic after surgical interventions [1] was compared to papaverine [2], pentazocine [3] and morphine [4]. Because it is a mixed agonist–antagonist with a long duration of action, buprenorphine is used in the treatment of heroin [5] and cocaine [6] addicts. Like other opiates it can be abused [7]. Buprenorphine has been suspected in the doping of racehorses [8]. Therefore, the matrices in which buprenorphine could be determined are very different, but especially pharmaceuticals and biological samples.

Several analytical methods for the determination of buprenorphine have been reported. The immunoassay techniques fluoroimmunoassay [9] and radioimmunoassay [8,10,11] have been developed for the determination of this drug in biological samples. Chromatographic techniques have been widely employed since they are powerful separation techniques. Although TLC was used for its determination in urine [12] and pharmaceutical preparations [13], GC and HPLC are the most common techniques. Mass spectrometry

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[8,14–16] and electron capture [17] are the most common detection systems used in conjunction with gas chromatography. Fluorescence [18] and mass spectrometry [19–21] have been combined with HPLC as detection systems.

On the other hand, electroanalytical techniques have been shown to be excellent for the determination of drugs in different matrices. The voltammetric determination of drugs in pharmaceutical products is by far the most common electroanalytical technique for pharmaceutical analysis. Many of the active constituents of formulations, in contrast to excipients, can be readily oxidised or reduced. Hence sample preparation usually consists of dissolving the active component from a particular formulation in a suitable solvent and performing a direct analysis on an aliquot of this solution. The selectivity of this method is normally excellent because the analyte can be readily identified by its voltammetric peak potential. Examples of the voltammetric determination of organic compounds in pharmaceuticals have been reported [22–25]. In this context, the existence of electroxidizable groups in the molecular structure of buprenorphine has been widely exploited for analytical purposes and is the basis of determinations by HPLC with electrochemical detection [26–29] employing a glassy carbon electrode. However, the coupling of chromatographic techniques to electrochemical detection increases the analysis time and it has to be taken into account that separation may not be needed for simple matrix samples. In these cases, a voltammetric determination is a valuable alternative.

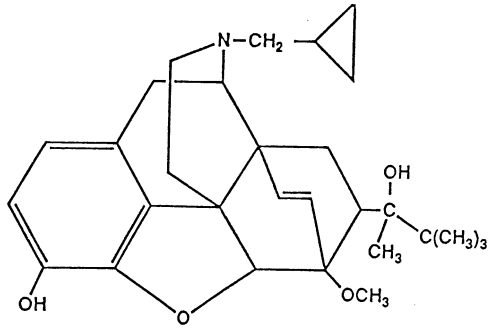

Fig. 1. Molecular structure of buprenorphine.

In this paper an electrochemical study of buprenorphine is presented as the basis for its determination in pharmaceuticals. A carbon paste electrode, that allows the total renovation of the surface and presents wider electroactivity limits and lower capacitive current than glassy carbon is employed as a working electrode. The voltammetric method proposed is applied to the quantitation of the drug in liquid and solid preparations.

2. Experimental

2.1. Reagents

Stock solutions of buprenorphine (Laboratorios Dr Esteve) were prepared in 0.1 M $HClO_4$ from the respective hydrochloride and remained stable all over the experiments. Carbon paste was prepared by mixing 1.8 ml of paraffin oil (Uvasol, Merck, Darmstadt, Germany) with 5 g of spectroscopic grade graphite powder (Ultracarbon, Dicoex, Bilbao). Britton–Robinson buffers covering the pH range 2–11 were used for pH dependence studies as well as 0.1 M $HClO_4$. Water was purified in a Milli-Q system. All other reagents were of analytical reagent grade.

2.2. Instruments

Cyclic and linear sweep voltammetry was performed by coupling a Metrohm VA-612 scanner to a Metrohm VA-611 potentiostat using the traditional three electrode potentiostat system. A home-made carbon paste electrode having a 0.13 cm^2 geometric area was used as the working electrode, while a platinum wire served as auxiliary electrode. Voltammograms were recorded on a Graphtech WX-4421 X–Y recorder.

Alternatively, hydrodynamic voltammetric measurements were carried out using a Metrohm E-506 polarograph coupled to a Metrohm VA-647 stand. In this case, a 0.07 cm^2 rotary glassy carbon electrode was used as working electrode.

Potentials were measured versus a saturated calomel electrode and versus a $Ag/AgCl/KCl_{sat}$ reference electrode.

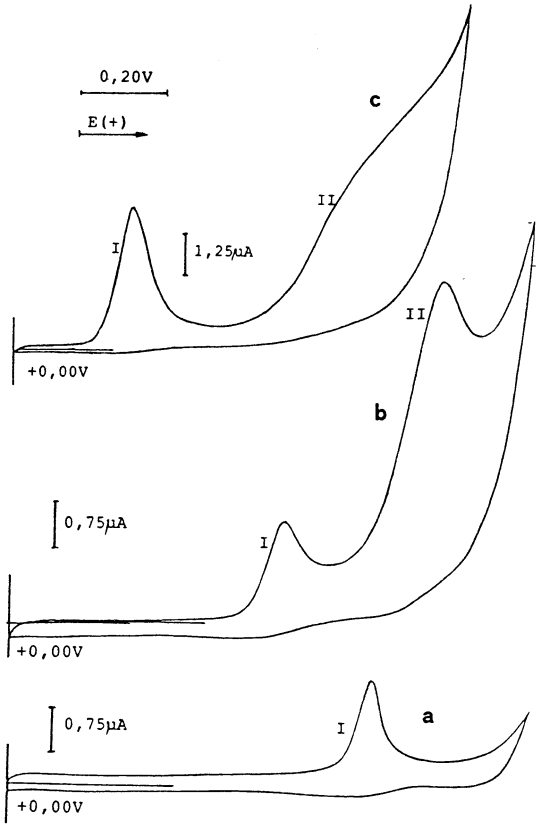

Fig. 2. Cyclic voltammograms obtained for a 5×10^{-6} M solution of buprenorphine in (a) 0.1 M $HClO_4$, (b) BR pH 4.03 and (c) BR pH 9.06, $v = 20$ mV s^{-1}.

2.3. Procedures

2.3.1. Electrode activation

The electrode was kept in a stirred solution (500 rev./min in 0.1 M $HClO_4$) for 20 s with electrolysis at + 1.55 V (vs SCE). Electrolysis and stirring were then switched off and the solution allowed to quiesce for 10 s. Finally, the starting potential (0.00 V) was maintained for 10 s before the potential scan was started.

2.3.2. Sample preparation

2.3.2.1. Vials. The vial content (1 ml) was quantitatively transferred to a 10-ml flask and made up with 0.1 M $HClO_4$. Aliquots of 100 μl were transferred to a polarographic cell containing 10 ml of 0.1 M $HClO_4$. After homogenization by stirring at 500 rev./min during 60 s the cyclic voltammograms were recorded from an initial potential of 0.00 V (vs SCE) at a scan rate of 20 mV s^{-1} to + 1.10 V. Quantitation of the drug was achieved by the standard additions method performing four additions. The electrode was cleaned following the procedure stated above before recording each voltammogram.

2.3.2.2. Tablets. A tablet was pulverised and dissolved in distilled water. After centrifugation to separate the insoluble components, the liquid fraction was quantitatively transferred to a 10-ml flask and made up with 0.1 M $HClO_4$. Aliquots of 100 μl were transferred to a polarographic cell containing 10 ml of 0.1 M $HClO_4$, following the procedure specified for vials.

3. Results and discussion

3.1. Voltammetric studies under semi-infinite diffusion conditions

The cyclic voltammetric behaviour of 5×10^{-6} M solutions of buprenorphine was examined with varying pH over a wide range of values from acidic (0.1 M $HClO_4$) to alkaline (pH 11). Different carbon paste was used for each measurement in order to avoid memory effects. In the entire pH range, a well-defined peak can be observed that can be ascribed to the irreversible oxidation of the aromatic hydroxy group (Fig. 2a). For pH values above four, a second process appears at more positive potentials (Fig. 2b), similar to that observed in the oxidation of the tertiary amino group of other molecules studied [26,30–33]. At high pH values the second process becomes undefined and vague (Fig. 2c) perhaps due to the inhibition caused by adsorption of products from the hydroxy group oxidation.

The peak potential of both processes moves to less positive potentials with increasing pH. The half-peak potentials vary from + 0.76 (pH 1) to + 0.09 V (pH 11) for the first and from + 0.91 (pH 4) to + 0.70 V (pH 9) for the second process. The following equations show the linear relation

existing between the half-peak potential and the pH:

$$E_{p/2}\ (\mathrm{V}) = 0.84 - 0.067\ \mathrm{pH},\ r = 0.9997,$$
$$n = 11,\ \mathrm{pH} = 1\text{–}11$$
$$E_{p/2}\ (\mathrm{V}) = 1.1 - 0.042\ \mathrm{pH},\ r = 0.9993,$$
$$n = 6,\ \mathrm{pH} = 4\text{–}9$$

The value of βn (β = charge transfer coefficient, n = number of electrons) was estimated through the $E_p - E_{p/2}$ measurement from the voltammogram recorded in 0.1 M $HClO_4$ using a scan rate of 20 mV s^{-1} with the equation for irreversible processes:

$$\beta n = 0.048/(E_p - E_{p/2})$$

where potentials are measured in volts units. The value obtained for βn was 0.68.

For the first process, the peak current increases with pH, this effect being stronger after pH 8. However, for the second process the effect is opposite, there is a decrease with pH, more notable at low pH values (Fig. 3).

The first process was chosen as indicative of the greatest analytical interest. The proximity of the second process to the supporting electrolyte and the possibility of electrode fouling by adsorption of products of the amino group oxidation are reasons for this choice. $HClO_4$ (0.1 M) was chosen as supporting electrolyte for the remainder of the work.

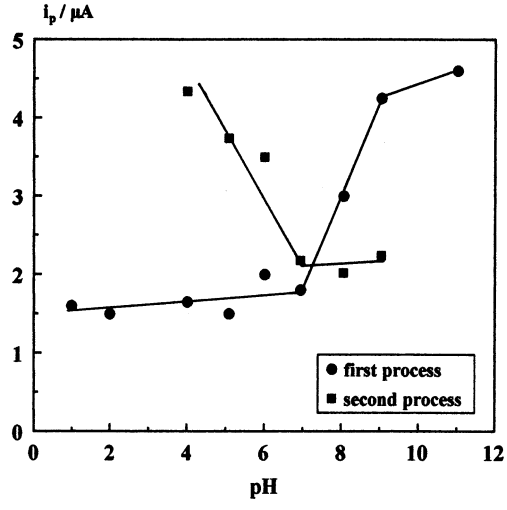

Fig. 3. Peak intensity dependence on pH; [buprenorphine] = 5×10^{-6} M, $v = 20$ mV s^{-1}.

The surface of solid electrodes is susceptible to fouling by the adsorption of products of the electrochemical process. This is an important drawback of voltammetry on solid electrodes. In the case of carbon paste electrodes, as reported previously [26,30–34], activation by high anodic potentials can be quite effective. At these potentials, background electrolysis occurs with evolution of oxygen that, in turn, causes oxidative changes in the graphite surface and displaces the adsorbed organic layer. In Fig. 4a, six successive voltammograms recorded without previous electrode pretreatment in a 1×10^{-5} M buprenorphine solution are presented. The decrease observed in the peak current comes from a decrease in the electroactive area. In order to clean the electrode, potentials between + 1.00 and + 1.60 V (vs SCE) were applied from 10 to 60 s to the electrode under stirring at 500 rev./min, recording, for each case, six voltammograms. Following the procedure described under experimental (+ 1.55 V, 20 s) the measured peak potentials and currents were highly reproducible (Fig. 4b). The R.S.D. of the peak current was 0.85% for a concentration level of 1×10^{-5} M ($n = 6$). The use of this procedure means that it is not necessary to renew the electrode surface every time a measurement is made thus decreasing analysis time and increasing precision.

The following experiment was made with the aim of elucidating the character of the buprenorphine first process and observing the effect that scan rate has on analytical signal. When scan rate is varied from 10 to 100 mV s^{-1} in a 5.0×10^{-6} M solution of buprenorphine, a linear dependence of the peak intensity upon the square root of the scan rate is found, demonstrating a diffusional behaviour. The equation is noted below:

$$i_p/\mu\mathrm{A} = 0.237\ v^{1/2}/(\mathrm{mV\ s^{-1}})^{1/2} - 0.169,$$
$$r = 0.9997,\ n = 10$$

The existence of an intercept could mean the presence of an adsorptive component in the first process of the buprenorphine. In order to confirm this, the linearity between log i_p and log v was studied. The equation obtained is:

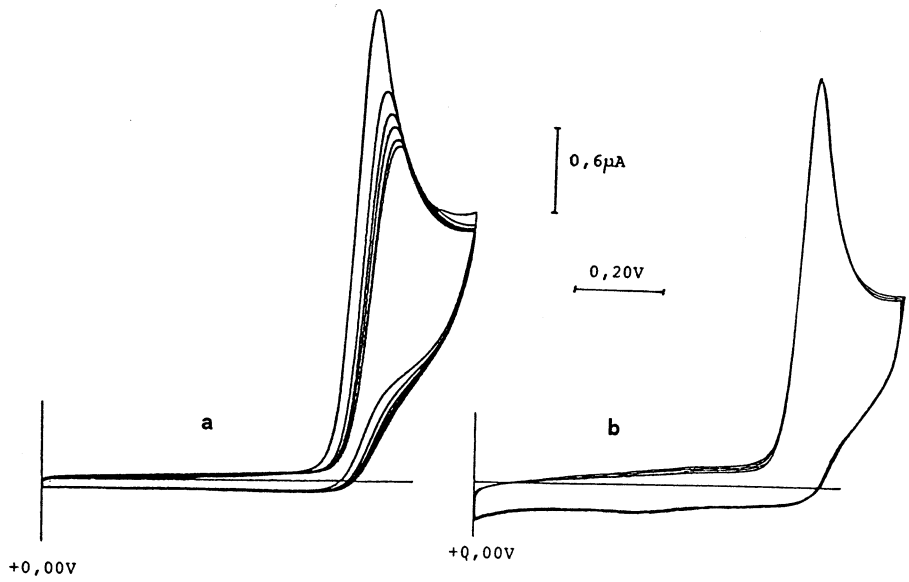

Fig. 4. Six successive voltammograms from a 1×10^{-5} M solution of buprenorphine recorded in 0.1 M $HClO_4$ (a) without and (b) with previous electrode pretreatment; $v = 20$ mV s^{-1}.

$$\log(i_p/\text{nA}) = 0.577 \log(v/\text{mV s}^{-1}) - 0.806,$$
$$r = 0.9995,\ n = 10$$

The slope deviation of the value predicted by the theory [35] (0.50) for irreversible and diffusional in nature processes, confirms the existence of this adsorptive component.

The relationship between the peak current and the concentration is linear from 5.0×10^{-7} to 1.0×10^{-5} M. The study was carried out using a scan rate of 20 mV s^{-1}. Linearity is expressed by the following equation:

$$i_p/\mu\text{A} = 2.4 \times 10^4\ C/\text{M} + 0.0073,$$
$$r = 0.9998,\ n = 11$$

The limit of detection, calculated as the concentration corresponding to three times the S.D. of the estimate, was found to be 2.0×10^{-7} M.

3.2. Studies under hydrodynamic conditions

Hydrodynamic voltammograms of buprenorphine for concentrations ranging from 5.0×10^{-6} to 1.0×10^{-5} M were recorded at several rotation speeds (from 500 to 3000 rev./min, that is from 8.33 to 50.00 Hz s^{-1} respectively). The data obtained show a good agreement with the Levich predictions [36]. The existence of linearity between i_p and $w^{1/2}$ (Fig. 5) demonstrates that the process is controlled by diffusion. The half-wave potential is $+0.73$ V vs $Ag/AgCl/KCl_{sat}$.

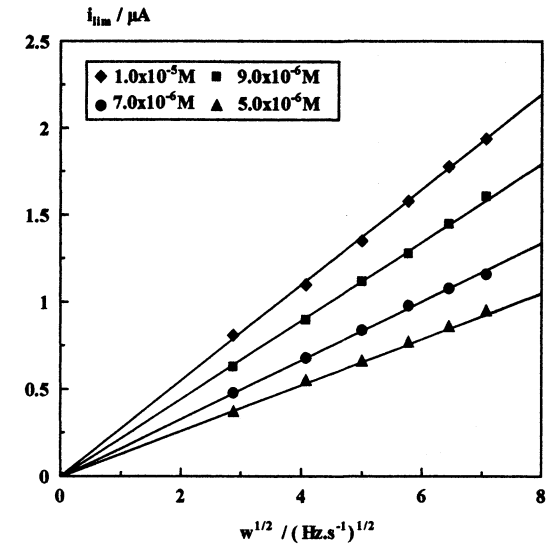

Fig. 5. Levich plots of buprenorphine; 0.1 M $HClO_4$, $v = 20$ mV s^{-1}.

Table 1
Results obtained for the voltammetric determination of buprenorphine in pharmaceuticals

Sample	Vials (mg ml^{-1})	Tablets (mg)
1	0.296	0.208
2	0.303	0.199
3	0.294	0.209
4	0.308	0.191
5	0.299	0.196
$X \pm \sigma_{n-1}$	0.300 ± 0.006	0.201 ± 0.007
Nominal value	0.302	0.206
Absolute error (mg ml^{-1})	0.002	0.005
Relative error (%)	0.66	2.4

Calibration graphs for buprenorphine at different rotation speeds are linear and when a rotation speed of 1500 rev./min is used, the limiting current is given by the equation:

$$i_{lim}/\mu A = 1.30 \times 10^5\ C/M - 0.0102,$$
$$r = 0.996,\ n = 5$$

3.3. Analytical application

The voltammetric response of buprenorphine was used for its determination in pharmaceuticals. The buprenorphine is commercially provided by Laboratorios Dr Esteve in two different presentations named BUPREX: liquid preparations (1 ml) nominally containing 0.300 mg and tablets (60.0 mg) containing 0.200 mg of buprenorphine. The liquid preparation contains dextrose (anhydrous, 0.05 g/ml) in aqueous solution and the tablet lactose (29.5 mg), mannitol (18.0 mg), cornstarch (9.0 mg), povidone (1.2 mg), citric acid (anhydrous, 0.9 mg), sodium citrate (0.4 mg), colloidal silicon dioxide (0.3 mg) and magnesium stearate (0.5 mg). Five vials and five tablets were analysed following the procedure described under Section 2. Well-defined peaks were obtained and no interferences were observed. The results obtained are given in Table 1. They compared favourably with those offered by Laboratorios Dr Esteve. A t-test was carried out to evaluate the absence of systematic errors comparing both values and taking the nominal value as the true one. Considering a level of confidence of 95% and four degrees of freedom it can be concluded that there are no systematic errors in this methodology.

4. Conclusions

The voltammetric behaviour of buprenorphine has been studied. Buprenorphine presents two processes depending on the pH. The first, caused by the oxidation of the phenolic group, is present at all pH values. The second process, due to the oxidation of the amino group appears between pH 4 and 9. The first process (in 0.1 M $HClO_4$) was used for the determination of buprenorphine. With a robust, simple and rapid pre-treatment of the electrode excellent reproducibility of the current signal is obtained. The electrochemical response shows linearity with concentration and was successfully applied to the quantitation in pharmaceuticals. The proposed method is also fast and cheap and could be used in quality control processes.

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