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A combined liquid three-phase micro-extraction and differential pulse voltammetric method for preconcentration and detection of ultra-trace amounts of buprenorphine using a modified pencil electrode

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ABSTRACT

A combination of polytetrafluorethylene membrane-based liquid three-phase micro-extraction and voltammetry was used for the micro-separation and determination of buprenorphine. Type of the organic solvent used, pH levels of the donor and acceptor phases, salt concentration, extraction time, stirring rate, and electrochemical parameters as the essential factors affecting the liquid three-phase micro-extraction of buprenorphine were investigated. Differential pulse voltammetry exhibited two linear dynamic ranges of 1.0–109.0 pmol L^{-1} and 0.109 nmol L^{-1}–0.11 μmol L^{-1} of buprenorphine and the detection limit was found to be as low as 0.6 pmol L^{-1} of buprenorphine. Also, the effects of a number of common substances potentially interfering with selectivity were studied. The results indicate that the proposed method is highly selective and sensitive for buprenorphine detection in real samples such as human urine and plasma of both drug-addict and non-addict human subjects.

1. Introduction

Buprenorphine (Fig. 1) is a strong semi-synthetic opiate pain killer with a sovereignty of 20–40 times higher than that of morphine [1]. It is commonly used at higher doses for treating opioid addiction and at lower doses for controlling both moderate to acute pains in non-opioid-tolerant individuals and moderate chronic pains [1]. Buprenorphine causes three major effects of diminished respiration, gladness, and reduced pain. In fact, at high doses and under certain circumstances, buprenorphine can not only block the effects of full opioid agonists but also precipitate with drawal symptoms [2]. As an analgesic, it has been successfully used via intramuscular, intravenous, or sublingual paths for appeasing moderate to severe and chronic pains [3]. Like other opiates, it has been reportedly abused [4] as in the doping of racehorses [5]. Therefore, the matrices in which buprenorphine could be defined are very different, especially in pharmaceuticals and biological samples.

Different methods have been reported for the determination of buprenorphine, including adsorptive stripping voltammetry [6], gas chromatography–mass spectrometry [7–10], radioimmunoassay [5], and HPLC [11–17]. As a powerful technique for the determination of pharmaceutical compounds in biological fluids, electrochemical techniques such as differential pulse voltammetry (DPV) can be considered as a suitable and sensitive alternative to other instrumental methods [18–22].

Liquid three-phase micro-extraction (LPME) has been recently used as a strong preparation method for preconcentration and cleanup [23–27]. In this method, the analyte is extracted from an aqueous sample (donor solution) through a thin layer of an organic solvent where it is immobilized within the pores of a porous membrane. Then, it is back-extracted into an acceptor solution inside an especially designed extraction cell. The membranes, being typically disposable, contribute to the elimination of sample carryover. In this technique, selectivity for the analyte considered may be affected by the type of the organic solvent used and the micro-extraction conditions [28,29].

In coupling an electrochemical method with LPME for trace analysis, type and shape of the working electrode affect the voltammetric results [30–32]. For this reason, different types and shapes of working electrodes have been tested in voltammetric methods, among which the multiwall carbon nanotubes-modified graphite electrode has proved to be a suitable one [32,33]. In this work, multiwall carbon nanotubes (MWCNTs) were used for modifying a pencil graphite electrode to develop a sensitive and useful tool for the determination of buprenorphine. The membrane that separated the two phases was typically a flat polytetrafluorethylene (PTFE) membrane or a tubular hollow fiber made of polypropylene with well-defined thickness, porosity, and pore size (usually 0.2–0.4 μm) [34]. PTFE membrane was laminated with a great variety of fabrics and paper [35,36]. This type of membrane is both hydrophobic and

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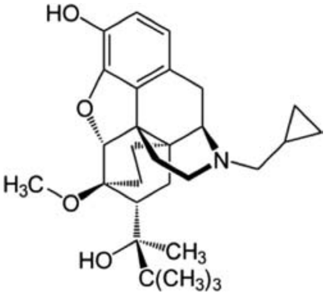

Fig. 1. Structure of buprenorphine.

inert not only to most organic solvents but also to acidic and/or basic solutions. In this experiment, a micro-pore membrane made of PTFE in 2-dimensions was used as the membrane medium for filtration.

Moreover, the combined PTFE–LPME and differential pulse voltammetry was used for the cleanup, preconcentration, and determination of buprenorphine at ultra-trace levels using a MWCNTs–modified pencil graphite electrode. In this method, buprenorphine is extracted and preconcentrated from the donor phase to the acceptor phase, which can, then, be oxidized at +0.75 V at the surface of the modified electrode to create the analytical signal. Due to its capability to clean up the analyte from other potential interfering compounds, the advantages of this include high sensitivity and selectivity for determining buprenorphine in real samples such as biological fluids at ultra-trace levels.

2. Experimental

2.1. Reagents

PTFE fiber membrane (with a pore size of 0.22 μm,) was obtained from Membrana (Wuppertal, Germany). Isoamyl benzoate, n-dodecane, propyl benzoate, dibenzyl ether, sulfuric acid (98% w/w, $d=1.84\ \mathrm{g\ cm^{-3}}$) and MWCNTs (20–30 nm in diameter, 5–15 μm long, and more than 90% pure) were obtained from Aldrich. Other reagents were of analytical grade, all obtained from Merck (Darmstadt, Germany). The water required for the experiments was produced by an ultrapure (18.2 mΩ $\mathrm{cm^{-1}}$ at 25 °C) purification system (Millipore, USA). The acceptor phase was prepared by dilution of conc. H_2SO_4 with water to get a solution with pH 2.0. The donor phase was prepared by dissolving NaOH in water to a pH level of 9.0.

2.2. Apparatus

All electrochemical measurements were performed in an analytical system (Micro-Autolab, Utrecht, The Netherlands) including a potentiostat/galvanostat connected to a three-electrode cell (Metrohm Model 663 VA stand), linked to a computer (Pentium IV, 1200 MHz) and using the micro-Autolab software. The system was run on a PC using GPES and FRA 4.9 software. A pH–meter (Corning, Model 140) with a double junction glass electrode was used to control the pH of the solutions. Scanning electron microscope (SEM) was performed using a Philips XLC.

2.3. Preparation of the microelectrodes

An Ag/AgCl reference microelectrode was made according to Ref. [24]. A piece of platinum wire (0.25 mm o.d.) was used as a counter electrode.

A pencil graphite electrode (0.7 × 90 mm) modified with MWCNTs was used as the working electrode. To produce uniform and stable films of MWCNTs at the surface of the electrodes, MWCNTs were initially dispersed in a suitable solvent and then immobilized on the surfaces of various substrates by physical or chemical methods. In this study, 0.005 g of activated MWCNTs was dispersed in 5.0 mL of DMF and the mixture was sonicated for 5 min. The pencil electrode was pretreated with 5 cycles in the cyclic voltammetry in 1.0 mol $\mathrm{L^{-1}}$ NaOH between 0 and 1.50 V. It was subsequently placed in the MWCNTs suspension for 45 min and dried at room temperature to prepare the required MWCNTs–modified graphite pencil electrode.

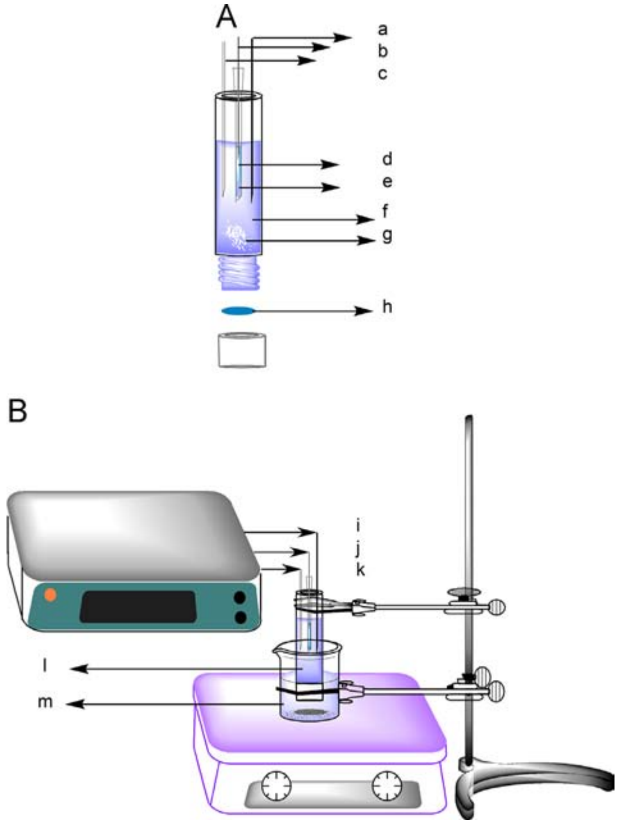

Fig. 2. Schematic of the of the (A): extraction cell containing (a): MWCNTs–pencil graphite electrode; (b): Ag-wire coated with an AgCl-thin film; (c): platinum wire microelectrode; (d): saturated KCl/AgCl solution; (e): agarose gel; (f): acceptor phase in the extraction cell; (g): extracted sample; (h): polytetrafluorethylene membrane. (B): Equipment used for PTFE–LPME in-situ differential pulse voltammetry containing the extraction cell and (i): working connection; (j): reference connection; (k): counter connection; (l): acceptor phase; and (m): donor phase.

2.4. PTFE membrane and extraction cell procedure

The PTFE membrane was initially cut into segments equal in size to that of the middle part of the extraction cell. The segments were then placed in a vial of acetone solvent. The membranes were removed from acetone and the acetone was allowed to evaporate completely. Before use, the membrane segments were completely immersed into propyl benzoate for 30 min for its pores to be impregnated. The extraction cell segments were then sonicated for 30 min in ethanol to remove any contaminants (Fig. 2A) and finally washed with acetone.

Extraction was performed according to the following procedure: 5.0 mL of the sample solution (pH 9.0) was filled into a 10 mL vial and set on a magnetic stirrer. The wet segment membrane was placed in the extracted cell and 200 μL of the acceptor phase (pH 2.0) was added to the extraction cell, which was subsequently immersed into the sample solution and the electrodes were incorporated into the extraction cell (Fig. 2B). Throughout the extraction process, the sample solution was continuously stirred (700 rpm) at room temperature for 55 min and in-situ voltammetric analysis was performed. A new piece of the membrane segment was employed for each extraction.

2.5. Voltammetric analysis

Once the extraction-electrochemical cell had been set up and the electrical connections checked, DPV was selected as a detection technique. DP voltammograms were recorded in the potential range of 0.00 to +1.50 V at a scan rate of 20 mV s^{-1} and a modulation amplitude of 80 mV. The peak current at the onset of the extraction was measured and recorded as a blank signal (I_b). The final signal was measured after 55 min and recorded as the sample signal (I_s). The difference between these currents ($\Delta I = I_s - I_b$) was considered as a net signal (ΔI) for each concentration. Calibration graph was prepared by plotting the net peaks current vs. the analyte concentrations in the solutions. Each experiment was repeated at least three times.

2.6. Real sample preparation

Urine samples were obtained from the Health Center of Isfahan University and stored in a refrigerator immediately after collection. The samples were then spiked right before separation and measurement. Using a 0.45 μm filter, 5.0 mL of the sample was filtered to be doubly diluted with the donor phase solution (pH 9.0). Then, 5.0 mL of the resulting solution plus 1.50 g NaCl was transferred into the donor phase section of the cell, and 200.0 μL of H_2SO_4 solution (pH 2.0) was transferred as the acceptor phase into the extraction cell. The donor phase was stirred at a rate of 700 rpm at room temperature for 55 min (the extraction time). Finally, voltammetric analysis was performed as described in Section 2.5 above. To check the accuracy of the proposed method, a high performance liquid chromatography (HPLC) [17], with a 4–μm NovaPak C_{18} (Waters) column (150 × 2 mm, ID) and a mobile phase of acetonitrile/acetate buffer (2.0 mmol L^{-1} at pH 3.0) (80:20, v/v; flow rate 200 μL min^{-1}; post column split 1:3) was used with a reflectance detector.

Human blood was obtained from the Health Center of Isfahan University. Blood plasma samples were stored in a refrigerator immediately after collection. Later, 5 mL of the plasma sample was mixed with 5 mL of 10% (w/v) trichloroacetic acid and centrifuged for 10 min at 4000 rpm. The samples were spiked right before separation and measurement. The solution thus obtained was doubly diluted with the donor phase solution (pH 9.0). Finally, 5.0 mL of the resulting solution was transferred with 1.50 g NaCl into the cell and the analysis was performed as described above (for the urine sample). The above HPLC method was also used to check the accuracy of the proposed method.

2.7. Calculation of the enrichment factor

The enrichment factor (EF) of buprenorphine was calculated using the following equation:

$$EF = C_{AP,final}/C_{DP,initial} \qquad (1)$$

where, $C_{AP,final}$ and $C_{DP,initial}$ are the final and initial concentrations of buprenorphine in the acceptor and donor phases, respectively. $C_{AP,final}$ was obtained from the calibration curve.

3. Results and discussion

3.1. Characterization of the modified electrode

A powerful LPME method for preconcentration and clean up of the analyte was used. The analyte was extracted from the donor phase into the organic solvent to be finally extracted into a smaller volume of an aqueous acceptor phase; hence, it was a kind of preconcentration method. As buprenorphine can be oxidized at the surface of the MWCNTs–pencil graphite electrode at a suitable applied potential, a voltammetric technique was, therefore, selected as a suitable analytical tool.

To prepare a uniform and stable film of MWCNTs at the surface of the graphite electrode, MWCNTs were initially dispersed in a suitable solvent before they were physically immobilized on the surface of the pencil graphite electrode. Among such solvents as N, N-dimethyl formamide (DMF), water, acetone, ethanol, and toluene, DMF is the most widely used dispersing solvent for preparing the

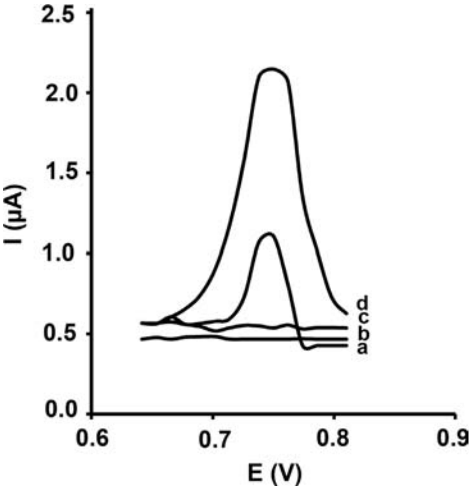

Fig. 3. Differential pulse voltammograms of unmodified pencil graphite electrode and MWCNTs-modified pencil graphite electrode in the blank solution at pH 2.0 (Fig. 3a and b, respectively) and in 40.0 nmol L^{-1} buprenorphine at pH 2.0. Extraction conditions: sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min.

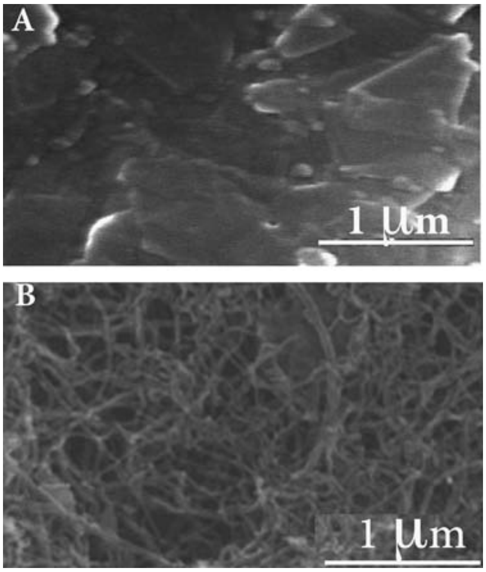

Fig. 4. SEM image of (A): unmodified pencil graphite electrode; (B) MWCNTs modified–pencil graphite electrode.

MWCNTs suspension. Therefore, MWCNTs were dispersed in DMF by sonication to fabricate the required MWCNTs–based pencil graphite electrode [33]. Fig. 3 shows differential pulse voltammograms of an unmodified pencil graphite electrode and the MWCNTs-modified pencil graphite electrode in solutions without buprenorphine, blank solution, (Fig. 3a and b, respectively) and with 40 ng mL^{-1} buprenorphine (Fig. 3c and d, respectively). These voltammograms show that the peak current of buprenorphine at the surface of MWCNTs-modified pencil graphite electrode (Fig. 3d) is much more than at the surface of unmodified pencil graphite electrode. This is due to the synergic effect of MWCNTs on the oxidation of buprenorphine at the electrode surface.

SEM pictures were captured to obtain detailed information about the surface structure and roughness of the working electrodes (Fig. 4). The roughness (thereby, the actual surface area) of the modified electrode (Fig. 4B) was far greater than that of the unmodified one (Fig. 4A). This helped us to have a smaller electrode size with a greater surface area in the cell.

The experimental conditions were optimized by investigating the effects of the organic solvent as well as the physical, chemical, and instrumental factors involved in the liquid three-phase microextraction and buprenorphine detection.

3.2. Selection of organic solvent

An important factor contributing to the efficient preconcentration of an analyte is the selection of an appropriate organic solvent for the PTFE membrane. The criteria used for this purpose include nonvolatility to prevent solvent loss during extraction, and immiscibility with water to serve as a barrier between the two donor and acceptor aqueous phases. Isoamyl benzoate, n-dodecane, propyl benzoate, and dibenzyl ether were evaluated in this experiment for the extraction of buprenorphine. For this purpose, 5.0 mL of 0.2 μmol L^{-1} buprenorphine (pH 9.0) was used. The PTFE membrane was impregnated with the organic solvent and the extraction cell was filled with the acceptor phase (aqueous solution, pH 2.0). The system was left for 55 min to complete the extraction process. The results presented in Fig. 5 indicate that propyl benzoate was the most suitable solvent among the ones used. As can be seen in Fig. 1, buprenorphine has an aromatic ring. Thus, the π-interaction between the organic solvent and the analyte could be regarded as the main reason for the suitability of propyl benzoate for extracting buprenorphine. It was, therefore, selected as a suitable organic solvent for further experiment.

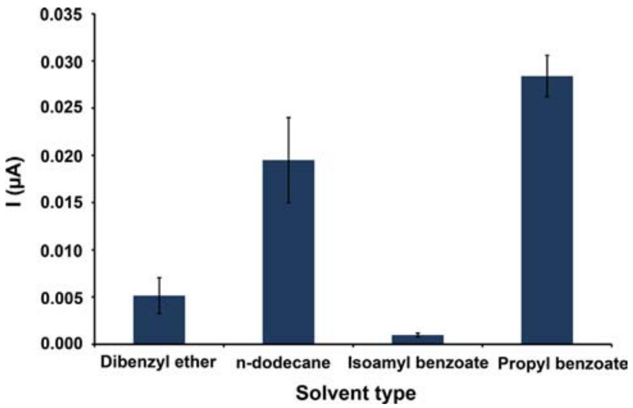

Fig. 5. Effect of different extraction solvents on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: buprenorphine, 0.2 μmol L^{-1}; Sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3).

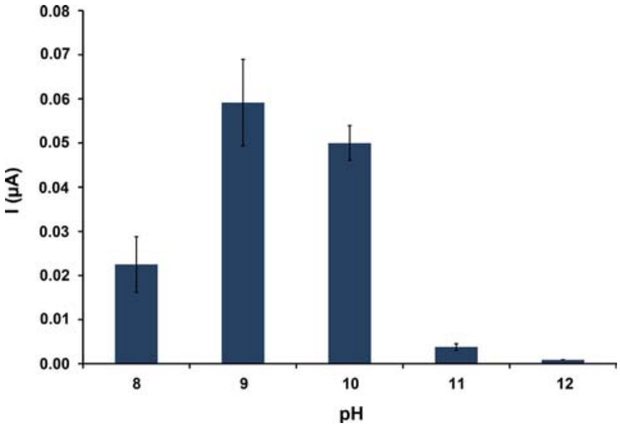

Fig. 6. Influence of basicity of the donor phase on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: buprenorphine, 0.2 μmol L^{-1}; sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3).

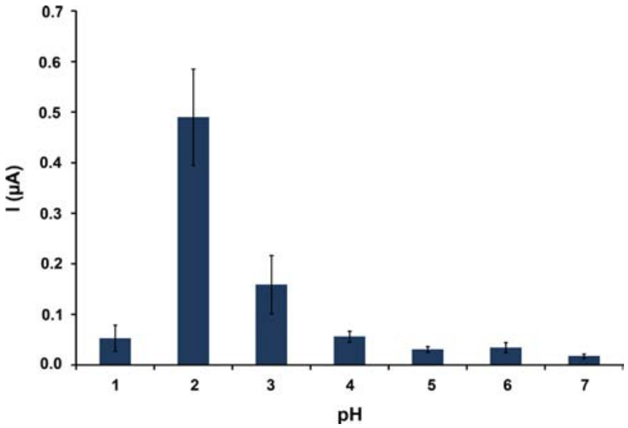

Fig. 7. Effect of acidity of the acceptor phases on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: buprenorphine, 0.2 μmol L^{-1}; sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3).

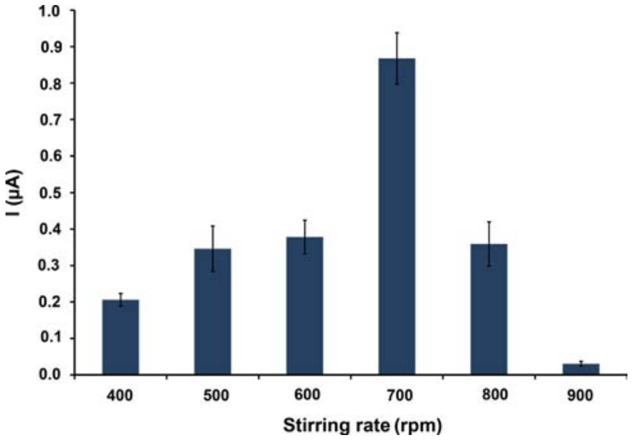

Fig. 8. Influence of stirring rate on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: buprenorphine, 0.2 μmol L^{-1}; sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3).

3.3. Influence of basicity and acidity of the donor and acceptor phases

The pH levels of the donor and acceptor phases play important roles in extraction efficiency. Hence, it is necessary to evaluate the analyte transferred from the donor into the organic phase. In this study, their effects were investigated in the pH range of 8.0 to 12.0 using NaOH solution. The results (Fig. 6) indicate that pH 9.0 produced the best signal. This is due to the fact that pK_{a1} of buprenorphine is 10.0; therefore, the major compound found at pH levels below 10 must have been the chargeless form of the molecule. Hence, this pH level was selected as the optimum value for the donor phase solution.

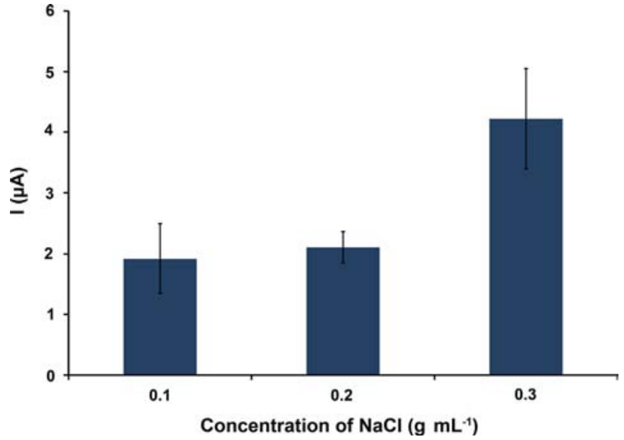

Fig. 9. Effect of salt on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: Buprenorphine, 0.2 μmol L^{-1}; sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3).

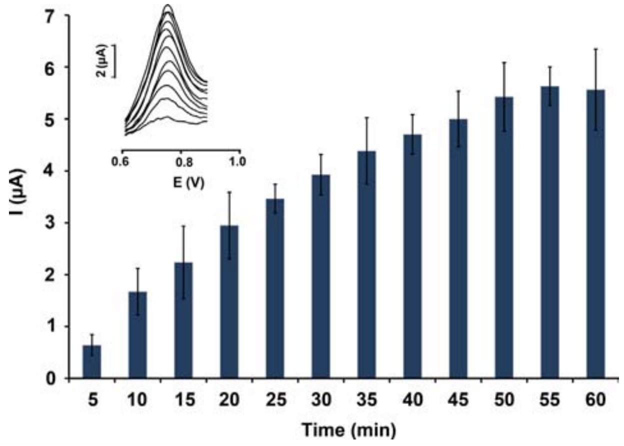

Fig. 10. Influence of extraction time on the extraction efficiency for PTFE–LPME and in-situ voltammetry. Extraction conditions: buprenorphine, 0.2 μmol L^{-1}; sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min (number of replications = 3). Inset shows the voltammograms of the sample solutions.

The influence of the pH level of the acceptor phase on the extraction efficiency was also investigated at levels between 1.0 and 7.0 using H_2SO_4 solution (Fig. 7). The results indicate that the best extraction efficiency was obtained at pH 2.0. This is due to the fact that the drug was protonated in the acidic solution and must have, therefore, been a charged molecule to be extracted in the aqueous solution. Therefore, H_2SO_4 solution with pH 2.0 was used as the acceptor phase.

3.4. Effect of stirring speed

Stirring of the donor phase solution led to increased rate of analyte diffusion from the donor phase into the acceptor phase, thereby decreasing extraction time. This indicated that the highest speed of the magnetic stirrer had to be selected. The drawback, however, was that air bubbles could have formed at high stirring speeds that would have prevented the analyte transfer into the extraction cell, thereby reducing extraction efficiency (Fig. 8). The best extraction in this experiment was achieved at a stirring rate of 700–rpm.

3.5. Effect of Ionic strength

Enhanced ionic strength of the sample solution reportedly affects extraction efficiency for many compounds in both liquid–liquid extraction [37] and solid phase micro-extraction [38]. The influence of ionic strength on the extraction efficiency was studied by adding different amounts of NaCl solution into the analyte solution in the range of 0–0.3 g mL^{-1} (Fig. 9). Based on the results obtained, the presence of 0.3 g mL^{-1} of NaCl improved the extraction of the analyte and, this salt concentration was, therefore, selected for the rest of the experiments.

3.6. Effect of extraction time

As extraction is an equilibrium process, it needs sufficient time to allow for the partitioning of the analyte between the donor and acceptor phases to occur. In order to study the effect of extraction time on the extraction efficiency, a series of experiments were carried out with different durations. In this study, due to the nature of the in-situ analysis, the voltammetric signal (peak current) was recorded every 5 min during a run so that the effect of extraction time on the performance of the method could be investigated in a single run (Fig. 10). The results confirmed that the equilibrium was reached between both phases after 55 min. So, this time was selected for the remaining experiments.

4. Analytical performance

Linear dynamic range, reproducibility, enrichment factor, and limit of detection (LOD) are figures of merit of the proposed method, as indicated by their evaluation for the extraction of

Table 1
Limit of detection, enrichment factor, and linear dynamic range of the proposed method.

Limit of detection (pmol L^{-1})	Enrichment factor	Dynamic range	Regression equation	R^2
0.6	25	1.0–109.0 pmol L^{-1} 0.109–110.0 nmol L^{-1}	$I(\mu A) = 0.001X + 0.053$ $I(\mu A) = 0.030X + 0.303$	0.997 0.994

buprenorphine from an aqueous solution under the optimum conditions. The results are summarized in Table 1. Calibration curves were obtained by plotting the net peaks current height vs. buprenorphine concentration in the aqueous sample. The results showed two linear segments with different slopes for buprenorphine concentration (Fig. 11).

The relative standard deviation (RSD%) was evaluated by extracting the analyte from four separate but identical samples and was found to be 5.7%. The LOD was found to be equal to 0.6 pmol L^{-1} buprenorphine as estimated based on $3S_{bk}/m$ (where, S_{bk} is the standard deviation for ten replicate signals of the blank solution and m is the slope of the calibration curve). Finally, an enrichment factor of 25 was obtained for the analyte.

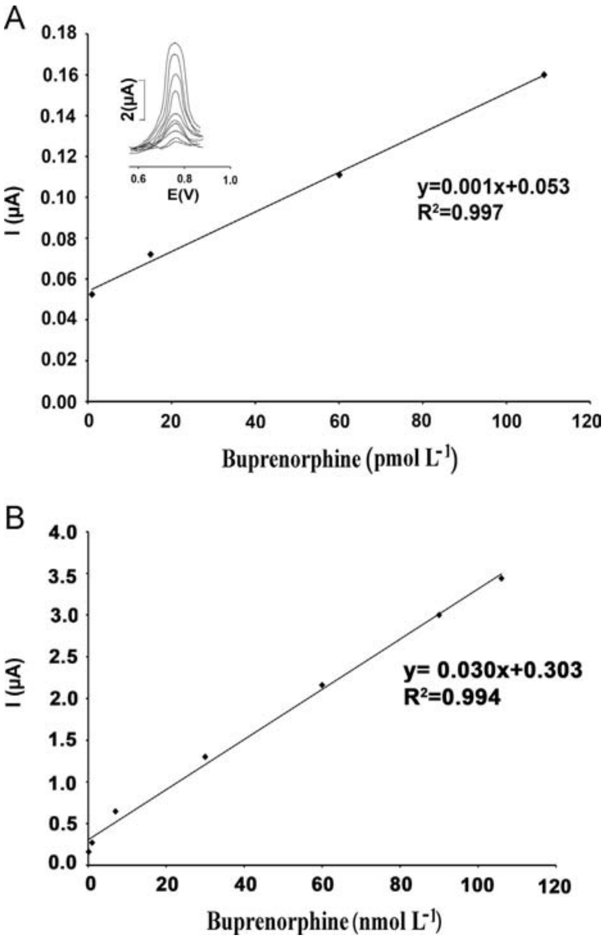

Fig. 11. Calibration curve for buprenorphine, (A) 1.0; 15.0; 60.0 and 109.0 pmol L^{-1}; (B) 0.109; 1.0; 7.0; 30.0; 60.0; 90.0 and 110.0 nmol L^{-1} buprenorphine. Inset: DP voltammograms of buprenorphine at different concentration levels. Extraction conditions: sample volume, 5.0 mL pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min. Inset shows the voltammograms of the sample solutions in different concentrations.

Table 2 shows the figures of merit of the proposed method compared to those for several published methods for buprenorphine determination. As shown, the proposed method is more sensitive than such expensive and time-consuming analytical ones as LC–MS–MS and radioimmunoassay methods [5,13].

5. Interference study

To evaluate the selectivity of the proposed method, the effects of several organic species on the determination of buprenorphine, which may be present in real samples, were investigated. To evaluate the method's selectivity, a standard solution of the analyte (12.5 nmol L^{-1} buprenorphine) plus different concentrations of several organic compounds such as thiourea, urea, uric acid, sucrose, fructose, glucose, and ascorbic acid were tested. The results showed that neither 1000–fold ascorbic acid, fructose, sucrose and glucose, and nor 800–fold uric acid, urea, thiourea and ascorbic acid affected the selectivity of the method proposed.

6. Real sample analysis

In order to assess the performance of the proposed method for the analysis of buprenorphine in complex matrices, its utility was investigated by determining buprenorphine in urine and plasma samples in both healthy and addict subjects. The accuracy of the method was also investigated using the HPLC method. The results presented in Table 3 confirm that the proposed method enjoys a good accuracy for the analysis of buprenorphine in real samples. Calibration curves were obtained for analysis of buprenorphine in urine and plasma samples as shown in Fig. 12.

7. Conclusion

In this study, a combination of micro-extraction using PTFE–membrane and in-situ DPV was successfully used for the analysis of ultra-trace amounts of buprenorphine in real samples in both healthy and drug-addict human subjects. Buprenorphine was extracted from real samples into the acceptor phase and analyzed in-situ using DPV. The results indicated that micro-extraction using PTFE–membrane could be used as an in-situ pretreatment procedure before electroanalytical analysis. The combination of micro-extraction and electrochemical techniques enhanced both selectivity and sensitivity for quantitative analysis of buprenorphine. The proposed method was also found to be more sensitive

Table 2
A comparison of the efficiency of the proposed method with several reported methods for the determination of buprenorphine.

Method	Limit of detection	Linear dynamic range	Reference
Radioimmunoassay	21.4 pmol L^{-1}	0.2–53.5 nmol L^{-1}	[5]
Adsorptive stripping voltammetry	0.02 μmol L^{-1}	0.01–0.8 μmol L^{-1}	[6]
Gas chromatography–mass spectrometry	0.1 nmol L^{-1}	0.4–42.7 nmol L^{-1}	[7]
Gas chromatography–mass spectrometry	0.5 nmol L^{-1}	0–0.1 μmol L^{-1}	[8]
GC–PCI–MS*	1.1 nmol L^{-1}	1.1–85.5 nmol L^{-1}	[9]
Liquid chromatography–mass spectrometry	0.1 nmol L^{-1}	0.2–213.8 nmol L^{-1}	[11]
LC–MS–MS	9.8 nmol L^{-1}	13.3–344.5 nmol L^{-1}	[13]
HPLC/Ionspray–MS	0.1 nmol L^{-1}	8.5–299.4 pmol L^{-1}	[17]
PTFE–LPME	0.6 pmol L^{-1}	1.0–109.0 pmol L^{-1} 0.109–110 nmol L^{-1}	This work

* gas chromatography–positive ion chemical ionization mass spectrometry (GC–PCI–MS).

Table 3
Determination of buprenorphine in biological fluids ($n=3$).

Samples	Added (mol L^{-1})	Found (mol L^{-1})	Recovery (%)	HPLC (mol L^{-1})
Urine 1	–	< Limit of detection	–	< Limit of detection
	4.0×10^{-10}	$(3.7\pm0.1)\times10^{-10}$	91.6	–
	1.0×10^{-10}	$(9.2\pm0.4)\times10^{-11}$	91.9	–
	2.5×10^{-9}	$(2.2\pm0.1)\times10^{-9}$	88.8	$(2.3\pm0.3)\times10^{-9}$
Urine 2	–	< Limit of detection	–	< Limit of detection
	1.0×10^{-10}	$(9.9\pm0.1)\times10^{-11}$	99.2	–
	1.0×10^{-9}	$(9.1\pm0.4)\times10^{-10}$	91.3	–
Plasma 1	–	< Limit of detection	–	< Limit of detection
	4.0×10^{-10}	$(3.8\pm0.2)\times10^{-10}$	96.8	–
	5.0×10^{-10}	$(4.7\pm0.4)\times10^{-10}$	81.5	–
	7.0×10^{-9}	$(6.8\pm0.3)\times10^{-9}$	90.1	$(6.5\pm0.7)\times10^{-9}$
Plasma 2*	1.0×10^{-10}	$(9.9\pm0.1)\times10^{-11}$	99.9	–
Urine 3*	–	$(3.2\pm0.1)\times10^{-8}$	–	$(3.1\pm0.3)\times10^{-8}$

* samples were taken from human urine and plasma of drug-addict.

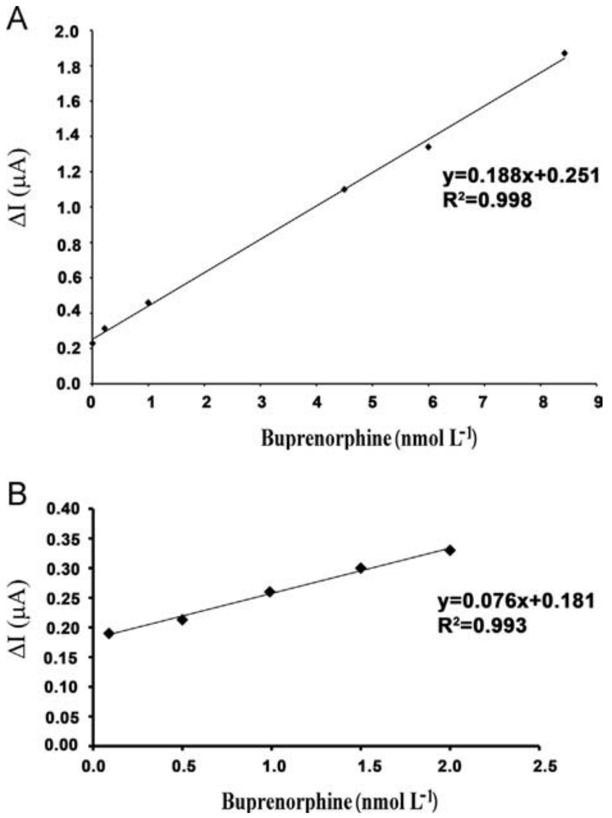

Fig. 12. Calibration curve for buprenorphine in real sample; (A) 0.005, 0.220, 0.998, 4.50, 6.00 and 8.50 nmol L^{-1} buprenorphine added to the plasma sample; (B) 0.09, 0.50, 0.99, 1.50 and 2.00 nmol L^{-1} buprenorphine added to the urine sample. Extraction conditions: sample volume, 5.0 mL at pH 9.0; acceptor phase, 200.0 μL at pH 2.0; organic phase, propyl benzoate; salt, 0.30 g mL^{-1} NaCl; stirring rate, 700 rpm at room temperature; extraction time, 55 min.

than such expensive and time-consuming analytical methods as LC–MS–MS and radioimmunoassay [5–13].

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