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# A new platform for sensing urinary morphine based on carrier assisted electromembrane extraction followed by adsorptive stripping voltammetric detection on screen-printed electrode

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ABSTRACT

Electromembrane extraction (EME) coupled with electrochemical detection on screen-printed carbon electrode has been developed for the quantification of morphine in urine samples. Charged morphine molecules were extracted from an aqueous sample by applying an electrical potential through a thin supported liquid membrane (SLM) into an acidic aqueous acceptor solution (20 μL) placed inside the lumen of a hollow fiber. Then, the acceptor solution was mixed with 20 μL of NaOH solution (0.1 M) and analyzed using screen printed electrochemical strip. Differential pulse voltammetry (DPV) peak current at 0.18 V was selected as the signal and the influences of experimental parameters were investigated and optimized using Box–behnken design and also one-variable-at-a-time methodology as follows: adsorptive accumulation time, 40 s; SLM, 2-nitrophenyl octyl ether + 10% tris-(2-ethylhexyl) phosphate + 10% di-(2-ethylhexyl) phosphate; pH of the sample solution, 6.0; pH of the acceptor solution, 1.0; EME time, 24 min; EME potential, 90 V and stirring rate, 1000 rpm. The calibration curve which was plotted by the variation of DPV currents as a function of morphine concentration was linear within the range of 0.005–2.0 μg $mL^{-1}$. The limit of detection and the limit of quantification were 0.0015 (S/N = 3) and 0.005 μg $mL^{-1}$, respectively. Finally, the proposed method was able to determine morphine simply and effectively at concentration levels encountered in toxicology and doping.



## 1. Introduction

Morphine, the main opium alkaloid, is used therapeutically for the treatment of acute and chronic pain in patients suffering from cancer or malignant diseases and also for relieving pain following surgical procedures.

Despite its pharmacological applications, morphine abuse for illegal purposes – as a doping or addictive drug – is one of the most serious forensic problems (Kolmonen et al., 2010; Spyridaki et al., 2006). Furthermore, morphine is the major metabolite of heroin and a minor metabolite of codeine. Therefore, developing sensitive and specific methods for the detection of morphine in biological fluids has become of great importance in forensic and clinical contexts such as drug abuse monitoring, diagnosis of poisoning and medicolegal death investigation (Mi et al., 2004).

Several methods have been reported for the determination of morphine in biological fluids including gas chromatography–mass spectrometry (Lee et al., 2006), micellar electrokinetic chromatography (Rodríguez et al., 2012), capillary zone electrophoresis (Tagliaro et al., 1998) liquid chromatography–mass spectrometry (Gustavsson et al., 2007), high-performance liquid chromatography with electrochemical detection (Mason et al., 1991), high-performance liquid chromatography with fluorescence detection (Venn and Michalkiewicz 1990), chemiluminescence (Barnett et al., 1993), radioimmunoassay (Chapman et al., 1994) and surface plasmon resonance (Sakai et al., 1998).

However, it is important to note that the mean concentration of morphine in biological fluids is in ng $mL^{-1}$ levels and sample preconcentration seems to be necessary prior to the determination step. Solid-phase extraction (SPE) (Botello et al., 2012), solid-phase microextraction (SPME) (Moller et al., 2010), solid phase microextraction membrane (SPMEM) (Yang and Xie 2004), surfactant enhanced liquid-phase microextraction (SE-LPME) (Sarafraz Yazdi and Es'haghi 2005), dispersive liquid–liquid microextraction (DLLME) (Shamsipur and Fattahi 2011) and monolith microextraction (Wei et al., 2006) have recently been developed for the preconcentration of morphine from various matrices.

As a novel cleanup and preconcentration technique, electromembrane extraction (EME) has recently gained favor and has

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been reported as a powerful technique for the selective extraction of analytes by applying an electrical voltage across the supported liquid membrane (SLM) (Gjelstad et al., 2009; Seidi et al., 2011). In EME, the ionized target analytes migrate from an aqueous sample (donor phase (DP)) through an organic solvent located in the pores of a porous hollow fiber into an aqueous acceptor phase (AP) inside the lumen of hollow fiber. The chemical nature of the SLM is highly critical for a successful and selective electrokinetic cross-membrane extraction. Basic analytes are successfully extracted with nitro aromatic solvents like 2-nitrophenyl octyl ether (NPOE) (Gjelstad et al., 2006), while, acidic compounds are extracted by aliphatic alcohols like 1-octanol (Balchen et al., 2007). It has also been demonstrated that the selective extraction of polar drugs in the presence of non-polar ones can be improved by means of applying carriers in the composition of SLM (Seidi et al., 2011). Thus, selective extraction can be obtained if an appropriate composition of SLM is chosen.

Electrochemical methods provide such advantages as easy preparation, low cost, short response time, low consumption of sample solution, relative high sensitivity, and good selectivity. As a result, there is an ever-growing interest to develop efficient electrochemical sensors for the determination of drug residuals in order to use in forensic applications (Radi 2010).

Replacing conventional electrochemical cells with miniaturized screen-printed electrochemical strips is of great interest in sensor development in many fields such as pharmaceutical, environmental and food analysis applications. In general, screen-printed electrodes (SPEs) provide a simple, user friendly, fast and inexpensive rout for electrochemical measurements. However, for the electroanalysis of drug residuals in biological fluids, especially in the case of drug abusing, sample cleanup and pretreatment as well as sample consumption are the main challenges. In other words, using EME prior to electroanalysis on screen printed strips can potentially overcome the drawbacks of low concentration of drug residuals, interferences of other electroactive biological species, fouling the electrode's surface, and sample amount limitation.

This paper presents a rapid, simple and inexpensive procedure for the selective extraction and quantification of morphine in the presence of other electroactive biological species (such as uric and ascorbic acids) using carrier assisted EME coupled with the electrochemical detection principles using SPEs. The results demonstrate that the proposed method provides a novel and simple way for the effective determination of morphine at the concentration levels encountered in toxicology and doping investigations of urine samples.

## 2. Experimental

### 2.1. Reagents and materials

Morphine sulfate (purity > 99.90%) was kindly donated by Tofigh Daru company (Tehran, Iran) and used without further purification. NPOE, 1-octanol, tris-(2-ethylhexyl) phosphate (TEHP) and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). Analytical grade NaOH and HCl were purchased from E-Merck (Darmstadt, Germany). HPLC grade water was prepared through a Milli-Q system (Millipore, Milford, MA, USA) and was used to prepare all the solutions.

### 2.2. Apparatus

All voltammetry experiments were performed using a μ-Autolab potentiostat/galvanostat type III with general-purpose electrochemical software (GPES version 4.7) (Ecochemie, Utrecht, the Netherlands). Three-electrode screen-printed electrochemical strips were provided by DropSens (Oviedo, Spain). The extractions were done by using an EPS-600Z d.c. power supply (Paya Pajohesh Pars, Tehran, Iran) with programmable voltage within the range of 0–600 V and current range of 0–0.5 A. Stirring the solutions was carried out by a Heidolph MR 3001 K magnetic stirrer (Schwa Bach, Germany).

### 2.3. Biological matrices and standard solutions

The urine sample was collected from a healthy volunteer with no recent history of drug-taking, and the sample preparation was performed immediately. A stock solution containing 1 mg $mL^{-1}$ of morphine was prepared in double distilled water, was stored at 4 °C and was protected from light. Sample solutions were prepared by diluting the stock solution with diluted urine (1:1/urine:water) samples.

### 2.4. EME procedure

The equipment for EME procedure is shown in Fig. 1. Platinum wires (with 0.2 mm diameter) were used as electrodes in the DP (4-mL sample solution) and the AP. The porous hollow fiber used for immobilizing the SLM and housing the AP was a PP Q3/2 polypropylene (Membrana, Wuppertal, Germany) with an internal diameter of 0.60 mm, 200 μm wall thickness and 0.2 μm pores. It was cut into 8.0 cm segments, cleaned in acetone and dried prior

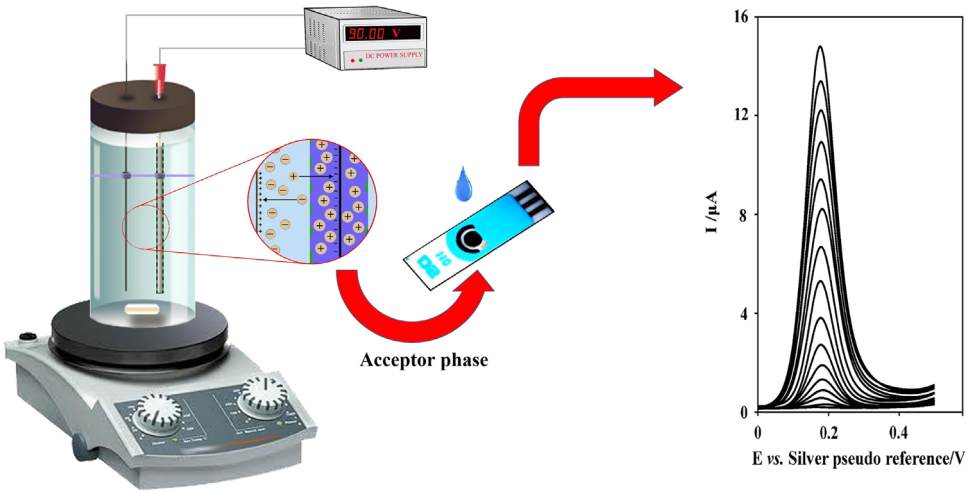


**Fig. 1.** Schematic illustration of the proposed carrier assisted EME–DPV method.