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Development of fast Fourier transformations with continuous cyclic voltammetry at an Au microelectrode and its application for the sub nano-molar monitoring of methyl morphine trace amounts

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Abstract

In this work, a new highly sensitive and fast method for monitoring methyl morphine in flow-injection systems is introduced. Mathematical methods such as signal integration and fast Fourier transformation used with continuous cyclic voltammetry. It should be stressed that this technique is simple, precise, accurate, time saving and economical. The effects of various parameters on the sensitivity of the detection system were examined. Eventually, it was concluded that the best condition was obtained within the pH value of 2.1, scan rate value of 80 V/s, accumulation potential of 400 mV and accumulation time of 0.4 s.

In addition, a special mathematical based numerical method is used for the calculation of the analyte signal and noise reduction. Where, the electrode response was calculated based on the partial and total charge exchanges on the electrode surface, after the background current subtraction from that of noise. In order to increase sensitivity of the method, the currents were integrated over a selected range of potential (including oxidation and reduction of the Au surface electrode), of the recorded CVs. The resulted signal was calculated based on changes in the charge in the CVs. The potential waveform, consisting of two potential steps for electrochemical cleaning, a step for accumulation and a potential ramp for current measurement, was applied to an Au disk microelectrode continuously.

In detail, the noteworthy advantages which this method illustrates in comparison with the other reported methods are the following; no oxygen removal is required from the test solution, a sub-nano molar detection limit and the fast determination of any such compound in a wide variety of chromatographic methods. Calibration curve is linear over the concentration range of 0.02–1.1 μM (5.98×10^3–329.2×10^3 pg/ml) ($r=0.997$) with a detection limit and a quantitation limit of 0.008 μM (2.39×10^3 pg/ml) and 0.01 μM (2.99×10^3 pg/ml), respectively. Consequently, the method illustrates the requisite accuracy, sensitivity, precision and selectivity to assay methyl morphine in its tablets.

Keywords: Methyl morphine; Ultra microelectrode; Fast fourier transformation; Continuous cyclic voltammetry

1. Introduction

Morphine derivatives, well-known potent narcotic analgesics and the active metabolite derived from heroin (3, 6-diacecylmorphine), have been reported to influence various immune functions. Codeine or the methyl ether derivative of morphine ((5alpha,6alpha)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol) is an alkaloid obtained from opium or prepared from morphine by methylation and occurs as white crystals and used in cough and cold preparations. In fact, methyl morphine is a common drug often offered to patients for pain relief from surgical procedure or carcinomatosis. Morphine is a μ-opioid agonist traditionally used for the treatment of moderate or severe pain [1]. The pharmacologic effects of methyl morphine are quite different from those produced by morphine when the drugs are administered systemically. Codeine is widely used in medicine and is less stringent than morphine in its action on the

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medullar centers. It is likewise much less addicting. Codeine has approximately one-third the activity of morphine on the cough center and approximately one-twentieth of morphine's activity on the higher centers of the brain. This property leads to wide prescription of codeine in cough syrups and complicates the work of the toxicologist since morphine and codeine react similarly with agents [1–4].

Many methods have been developed for the detection and determination of opiate derivatives, high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [5–11], fluorescence (FL) detection [12,13], combined electrochemical detection (ECD)–UV detection [14–16], and combined ECD–FL detection [17,18], Gas chromatography–mass spectrometry (GC–MS) [19,20], direct fluorimetric [21] chemiluminescence [22,23], capillary electrophoresis [24,25] and ion mobility spectrometry [26,27]. GC–MS is still the most widely used reference method but liquid chromatography coupled single-stage or tandem mass spectrometry (LC–MS, LC–MS–MS) is becoming increasingly important for the identification and quantification of analytes [28–34].

Voltammetric techniques are generally rapid and economical in the determination of some organic and inorganic compounds in aqueous systems with a sensitivity range of parts-per-billion. In fact, because of the selective detector, voltammetric techniques are useful for the samples. Additionally, owing to the movement of the analyte zone in an electrochemical flow cell for flowing solutions, the application of these techniques requires fast analyte accumulation and fast potential sweeping (which is not appropriate for large electrodes) [35,36]. Using voltammetric techniques have been further stimulated by the advent of ultra micro electrodes (UMEs), due to their steady state currents, higher sensitivity, increased mass transport and their ability to be used in electroanalysis in solutions with high resistance [37]. UMEs, for example, have been applied as sensors in various techniques such as flow injection analysis [38–50], cardiovascular monitoring and organic compounds analysis [51–53]. This research describes a new electrochemical method, based on flow injection analysis (FIA) and fast Fourier transform cyclic voltammetry (FFTCV), for the determination of methyl morphine.

2. Experimental section

The analytical grade reagents for the preparation of the eluent solution for the flow-injection analysis (0.05 M H_3PO_4) and 1 M NaOH (for the pH eluent adjustment), were obtained from the Merck Chemicals. The Drug Quality Control Center (Tehran, Iran) provided methyl morphine kindly as a gift. Also, methyl morphine tablets with a label of 15 mg codeine phosphate were purchased from a local pharmacy.

Furthermore, all solutions were prepared in double-distilled deionized water, filled with the background electrolyte solution and they were used without the removal of the dissolved oxygen.

2.1. Background electrolyte (BGE)

The running buffer or BGE was made by phosphoric acid (85% w/v) addition of 8.7 ml into a 1000 ml volumetric flask and dilution to a constant volume with distilled water. The pH was adjusted to the value of 2 with sodium hydroxide. The solutions were freshly prepared and filtered, using a Millipore filter (0.45 μm) each day.

2.2. Standards and sample solutions

2.2.1. Standard stock solutions

A methyl morphine standard stock solution 1.0×10^{-4} M was prepared from distilled water. This solution was light-protected using foil and stored at 4 °C for 5 days. It was found to be stable during this period.

2.2.2. Standard solutions for FIA

Aliquots of the standard stock methyl morphine solution were dispensed into 10 ml volumetric flasks. These flasks were filled up with the running buffer to give final concentrations range of 0.02–12.3 μM (5.98×10^3–3681.3×10^3 pg/ml).

2.3. Assay of sample preparation

Twenty tablets were weighed, finely powdered and portions equivalent to 15 mg methyl morphine were transferred into a 100 ml volumetric flask; 50 ml of distilled water were added, shaken thoroughly to dissolve, filled up and mixed well. Suitable solution aliquots were filtered through a Millipore filter (0.45 μm). 1 ml of the filtered solution was diluted with distilled water in a 100 ml volumetric flask. Then, 1 ml of the resulting solution was added to a 100 ml volumetric flask and filled up with 0.05 M phosphoric acid, reaching an initial concentration of 5.0×10^{-2} μM.

2.4. Determination of methyl morphin in human urine and plasma

For the determination of methyl morphin in human urine, 1 ml of untreated urine containing 10 ng/ml methyl morphin was placed into a 50 ml volumetric flask and diluted with pH 2 buffer solution to the mark. Then 50 μl aliquot was injected into the FIA system.

For the determination of methyl morphin in plasma, 100 μl aqueous methyl morphin solutions (5 ng/ml) were added to 100 μl of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 20 μl perchloric acid ($HClO_4$ 20%). After that, the mixture was vortexed for a further 30 s and then centrifuged at 6000 rpm for 5 min. Then 50 μl aliquot of the obtained supernatant was injected into the FIA system.

The voltammograms were recorded according to the above recommended procedure. The voltammograms of samples without methyl morphin do not show any signal that can interfere with the direct determination, so external calibration can be used.

2.5. Electrode preparation

The construction of the gold UMEs (12.5 μm in radius) involved the metal micro-wires (Good fellow Metals Ltd., UK)