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Fast Fourier transformation with continuous cyclic voltammetry at an Au microelectrode for the determination of morphine in a flow injection system

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Abstract

For the fast morphine monitoring in flow injection systems a highly sensitive method is being introduced in this work. The fast Fourier transformation with continuous cyclic voltammetry (FFTCV) in a flowing solution as a detection system was applied for the prompt morphine monitoring. Here it should be stressed that this technique is simple, precise, accurate, time saving and economical. This research includes the observation of the effects of various parameters on the sensitivity of the detection system. Eventually, it was concluded that the best condition was obtained within the pH value of 2, scan rate value of 40 V s^{-1}, accumulation potential of 400 mV and accumulation time of 0.6 s.

In detail, the noteworthy advantages which this method illustrates in comparison with other reported methods are the following; no necessity for the oxygen removal 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.

The method proved to be linear over the concentration range of 285–305,300 pg mL^{-1} ($r = 0.999$) with a detection limit and a quantitation limit of 95.5 and 285 pg mL^{-1}, respectively. Consequently, the method illustrates the requisite accuracy, sensitivity, precision and selectivity to assay morphine in its tablets and biological fluids.

Keywords: Morphine; Fast Fourier transformation; Continuous cyclic voltammetry; Ultra microelectrode

1. Introduction

Morphine, a well-known potent narcotic analgesic and the active metabolite derived from heroin (3,6-diacecylmorphine), has been reported to influence various immune functions. In fact, morphine is an alkaloid and a common drug often offered to patients for pain relief from surgical procedure or carcinomatosis. Morphine (MOR) is a μ-opioid agonist traditionally used for the treatment of moderate or severe pain [1]. It is extensively metabolized to its morphine-3-gluc-uronide (M3G), with normorphine (NM) and morphine-6-glucuronide (M6G) as minor metabolites, irrespective of the species or administration route [2–4].

Different methods have been developed for the detection and determination of opiate derivatives, including GC–MS [5–7], high performance liquid chromatography (HPLC) [8–11], chemiluminescence [12,13], direct fluorimetric [14], capillary electrophoresis [15,16] and ion mobility spectrometry (IMS) [17,18] and electrochemical methods [19]. Ion mobility spectrometry is a well-known technique, which offers low detection limit, fast response, simplicity and portability. Gas chromatography–mass spectrometry (GC–MS) is still the most widely used reference method but liquid chromatography coupled with single-stage or tandem mass spectrometry (LC–MS, LC–MS–MS) is becoming increasingly important for the identification and quantification of the analytes [20–23], especially for

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the more polar, thermo labile or low-dosed drugs, as indicated by Maurer [24].

Regarding the pharmacokinetic studies, various assay methods have been developed. Radio-immunoassay methods [25] present high sensitivity but, at the same time, they may demonstrate low specificity owing to the cross-reactivity among morphine, M-3-G and M-6-G. Furthermore, high performance liquid chromatography methods are vastly applied and allow simultaneous analysis of morphine and its glucuronides, detected by ultraviolet (UV) detection [26–29], fluorescence (FL) detection [30,31], combined electrochemical detection (ECD)–UV detection [32–34] and combined ECD–FL detection [35,36]. Nevertheless, UV and FL detection were not sensitive enough for morphine and its glucuronides in biological samples. Therefore, these compounds could be precisely detected by ECD. However, combined chromatographic ECD consumes long time to determine. Finally, mass spectrometric methods [37–41] have achieved the desired sensitivity and selectivity.

As far as voltammetric techniques are considered, they 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. Indeed, because of the selective detector, voltammetric techniques are useful for the samples. In addition, 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) [42,43]. The use of voltammetric techniques have been further stimulated by the advent of 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 [44,45]. UMEs, for instance, have been applied as sensors in various techniques such as flow injection analysis [46–47], cardiovascular monitoring and organic compounds analysis [48,49]. Here, this research describes a new electrochemical method, based on FIA and FFT Cyclic voltammetry, for the determination of morphine.

The instrumentation that we used in the previously reported papers was not able to determine this drug at this LOD level. For this work, we used the highly developed version of an old electrochemical system (the computer program is more advanced in the algorithm for integration and filtering). As a consequence, the noise level was less, resulting in a better S/N. Fortunately; it was feasible to determine morphine at a detection limit lower than those of other reported methods.

Furthermore, it should be noted that this novel electrochemical method has many advantages over the classical methods. For example, one of its most important abilities is the monitoring of several surface active compounds in a very short time. It means that 60 samples per hour can be determined. Currently, we are trying to explore its capability in new areas for the determination of significant compounds, which cannot be measured at very low concentrations by other methods. For instance, the method can be used in the indirect mode for the detection and determination of components of electroactive and non-electroactive substances.

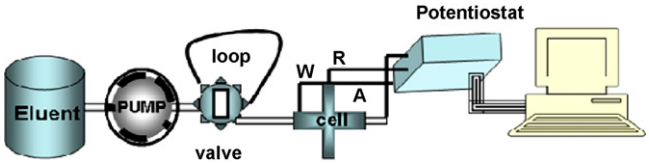

Fig. 1. Diagram of the flow injection setup.

2. Experimental

2.1. Flow injection setup

A 10-roller peristaltic pump (UltrateckLabs Co., Iran) and a four-way injection valve (Supelco Rheodyne Model 5020) with a 50-μL sample injection loop consisted of the necessary equipment for the flow injection analysis. The schematic of flow injection setup has shown in Fig. 1. Flow rate of the pump was set at 3 mL min^{-1} during an experimental run. The solutions were introduced into the sample loop by a plastic syringe. After, opening the loop in the way of eluent flow, the sample can pass over the working electrode. The potentiostate applies a potential in the range of gold oxidation in acidic media (Eq. (1)) and the potential waveform was shown in Fig. 3. The special program on computer can monitor the cyclic voltammograms online and every change on current or charge will be shown in program. In Fig. 2, the electrochemical cell of the flow injection analysis was shown in detail.

2.2. Reagents

For the experimental part of the research, the analytical grade reagents and the reagents for the preparation of the eluent solution for the flow injection analysis (0.05 mol L^{-1} H_3PO_4) and 1 mol L^{-1} NaOH (for the pH eluent adjustment), were obtained from the Merck Chemicals. The Drug Quality Control Center (Tehran, Iran) provided morphine kindly as a gift. Also, morphine tablets with a label of 15 mg morphine sulfate were purchased from a local pharmacy.

All solutions were prepared in doubly distilled deionized water, filled with the background electrolyte solution and they were used without the removal of the dissolved oxygen.

2.3. Background electrolyte (BGE)

The running buffer or BGE was made by phosphoric acid (85%, w/v) with the addition of 8.7 mL into a 1000 mL volumet-

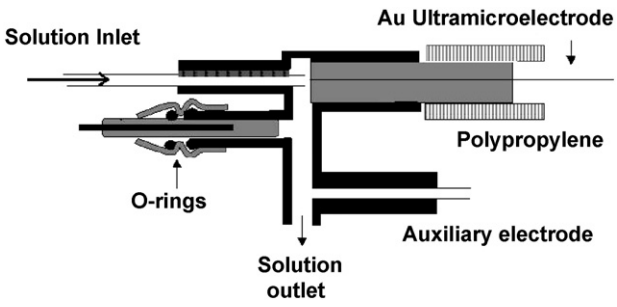

Fig. 2. Diagram of the electrochemical cell.