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# Flow injection analysis with amperometric detection of naltrexone in pharmaceuticals

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**Abstract**

Flow injection analysis (FIA) with amperometric detection using a carbon paste electrode is applied to the determination of naltrexone. The sample solution was injected into the carrier stream of 0.1 M perchloric acid, being determined by oxidation at +1.0V vs. Ag/AgCl/sat. KCl using a flow rate of 4 ml $min^{-1}$. A relative standard deviation of 1.5% was calculated for a concentration level of $10^{-5}$ M ($n = 17$) without carrying out a carbon paste electrode pretreatment. Calibration curves were found to be linear between $2 \times 10^{-8}$ and $10^{-5}$ M (almost three orders of magnitude) and the method has a detection limit of $2 \times 10^{-8}$ M. A simple and reproducible procedure is proposed for the determination of naltrexone in pharmaceuticals. The results compared favourably with those obtained by an HPLC-UV method. © 1997 Elsevier Science B.V.

*Keywords:* Flow injection amperometric analysis; Carbon paste electrodes; Naltrexone; Pharmaceuticals

## 1. Introduction

Naltrexone, a cyclopropyl derivative of oxymorphone is a potent, long acting and orally effective, narcotic antagonist. It is considered a useful adjunct for the maintenance of abstinence in the detoxified opioid addicts [1,2] when administered in selected patient groups and in combination with appropiate support mechanisms and psychotherapy. Therefore, it is a promising alternative to methadone and to drug-free approaches in the treatment of narcotic addiction.

Naltrexone is presented in pharmaceuticals either in liquid or solid form, being the quality control carryed out by using chromatographic techniques. These techniques, powerful separation techniques, are widely employed in its determination in a variety of matrices: urine, plasma, saliva [3–5]. However, they are time consuming and separation may not be needed for simple matrix samples. Electroanalytical techniques have been shown to produce excellent results in the field of analysis of drugs of abuse and related molecules [6–9] due to their simplicity, low cost and relatively short analysis time when compared with the above mentioned techniques.

In a previous paper [10], the electrochemical behaviour of naltrexone on carbon paste electrodes has been described. It gives rise to an irreversible anodic process that can be ascribed to

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the oxidation of the aromatic hydroxy group. In addition, a second process with a peak potential more positive appears at pH values above 5, due to the oxidation of the tertiary amin group. The electrooxidation of naltrexone has been used for its determination in pharmaceuticals.

Flow injection analysis (FIA) has been applied to the determination of pharmaceuticals and related drugs [11,12]. The possibility to automate many methodologies saving time by increasing sample capacity, has converted FIA to an analytical tool of great interest [13,14].

The use of amperometric detection is adequate for the quantitation of electroactive substances in simple matrices; therefore, it could be possible to determine naltrexone directly in pharmaceuticals.

In this paper, an automated method with electrochemical detection is proposed as an attractive and valuable analytical alternative for the determination of naltrexone in pharmaceuticals. The procedure described is fast, cheap and simple.

## 2. Experimental

### 2.1. Reagents

Stock solutions of naltrexone (Sigma, St. Louis, MO 63178) were prepared in 0.1 M perchloric acid from the respective hydrochloride. Carbon paste was prepared by mixing 1.8 ml of paraffin oil (Uvasol, Merck, D-6100 Darmstadt, Germany) with 5 g of spectroscopic grade graphite powder (Ultracarbon, Dicoex, Bilbao). Water was purified in a Milli-Q system (Millipore). All other reagents were of analytical reagent grade.

### 2.2. Apparatus

Cyclic voltammetry was performed by coupling a Metrohm VA-612 scanner to a Metrohm VA-611 potentiostat and a Linseis LY-1600 X-Y recorder and using the traditional three electrode potentiostat system. A home-made carbon paste electrode having a 7.1 $mm^2$ geometric area was used as the working electrode, while a platinum wire served as auxiliary electrode. Potentials were measured versus a silver/silver chloride/saturated potassium chloride reference electrode.

Flow injection amperometric measurements were performed using a twelve cylinder Perimax 12-Spetec peristaltic pump and a six-port rotary valve (Rheodyne 5020). Amperometric detection was carried out in a home-made thin-layer flow cell (Kissinger design) equipped with a carbon paste electrode of 7.1 $mm^2$ of geometric area. A downstream compartment coupled to the thin-layer cell outlet was put in place containing the reference electrode (silver/silver chloride/saturated potassium chloride) with a low resistance liquid junction, and a stainless steel waste tube acting as auxiliary electrode. A Metrohm VA E611 potentiostat was used as amperometric detector and its output signals were recorded on a Linseis L6012B strip chart recorder.

### 2.3. Analytical procedure

Aliquots of 13 $\mu$l of pharmaceutical (presented as a 20 ml liquid solution) are made up to 25 ml with 0.1 M $HClO_4$. Signals for triplicate injections (100 $\mu$l volume) are recorded using a detection potential of +1V vs Ag/AgCl/sat. KCl and a flow rate of 4 ml $min^{-1}$. Quantitation is achieved by a calibration curve.

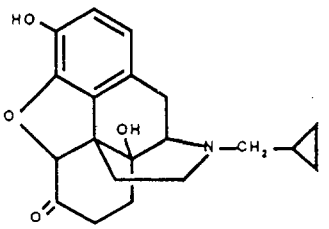
## 3. Results and discussion

### 3.1. Flow injection amperometric detection of naltrexone

Flow parameters were optimised prior to electrode calibration in order to obtain the best response. A single stream system was used and a coiled tube (50 cm in length, 0.8 mm i.d.) ensured the mixing of the sample and the carrier.

Naltrexone (Fig. 1) presents a well-defined and easily measurable peak in 0.1 M perchloric acid as demonstrated by preliminary experiments [10]. Therefore, this buffer has been chosen as the carrier stream for the remainder of the work.

The first parameter optimised in the FIA method was the working elecrode potential. This was made by injecting successive samples of 100 $\mu$l naltrexone ($10^{-5}$ M) into the carrier stream and varying the potential between +0.75 and




**naltrexone**

Fig. 1. Molecular structure of naltrexone.

+ 1.15 V. Fig. 2 shows peak current as a function of working potential in 0.1 M perchoric acid. It can be seen that peak current increases until it becomes independent of the working potentials at high potential values. A detection potential of + 1.0V vs. Ag/AgCl/sat. KCl was chosen as the optimum since the peak intensity is due to naltrexone oxidation only. The increase in the baseline is very small, which indicates that no background oxidation occurs at this potential.

Another parameter optimised was the flow rate of the 0.1 M $HClO_4$ carrier stream. Peak height and peak width depend largely on this parameter because the dispersion of the sample during its passage from the injection point to the detector depends on the time taken (the higher the flow rate, the smaller the time and the dispersion and therefore higher intensities are obtained). This effect was examined for a naltrexone concentration of $10^{-5}$ M and a detection potential of + 1.0 V by varying this parameter from 0.5 to 5 ml $min^{-1}$. Peak intensity increased linearly with the flow rate and simultaneously the peak width decreased. A flow rate of 4 ml $min^{-1}$ was used for further studies.

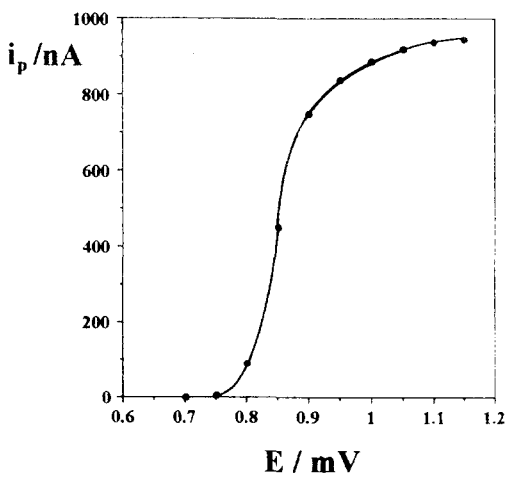



Fig. 2. Hydrodynamic voltammogram for the oxidation of naltrexone. Conditions: (naltrexone) = $10^{-5}$ M, carrier stream = 0.1 M $HClO_4$, flow rate = 3 ml $min^{-1}$, injection volume = 100 μl.

Using working conditions of + 1.0 V and a flow rate of 4 ml $min^{-1}$ the current reached its baseline value about 40 s after injection, allowing a maximum sampling frequency in the FIA system of about 90 samples $h^{-1}$.

Voltammetry at solid electrodes has the inconvenience of presenting adsorption processes and therefore the lack of a renewable electrodic surface. In the case of a porous material such as a carbon paste electrode, chemicals can penetrate a few microns into the electrode surface. In fact, successive records of the naltrexone oxidation process on the same electrodic surface produces a significant decrease in the analytical signal, probably due to an accumulation of products from the electrochemical reaction, which reduces the electrode active surface. To obviate this problem several cleaning procedures have been developed: mechanical, chemical or electrochemical [15]. The first one consists of polishing the electrode surface, the second one involves the treatment with a cleaning solution (its composition depending on the nature of the electrode and the impurity) and the last one tries to desorb compounds by applying either very high or low potentials to the electrode at a fixed time. The more drastic procedures require longer equilibration times before analytical work can be done. When using voltammetric detection for determining naltrexone [10], and taking into account that one should use the procedure which causes the least amount of physical damage to the surface of the working electrode, an electrochemical pretreatment of the carbon paste electrode at high positive potentials is employed. This process means that it is not necessary to renew the electrode surface every time a measurement is made, thus decreasing analysis time and increasing precision. In the case of a flow system, the possible surface poisoning

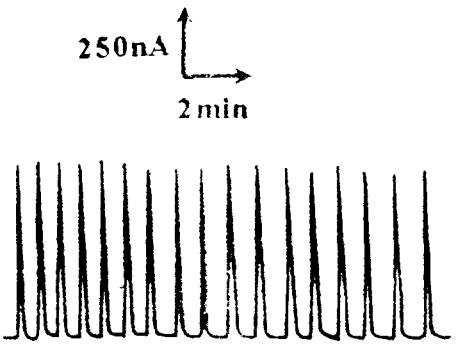



Fig. 3. Successive FIA signals corresponding to a $10^{-5}$ M naltrexone solution. Conditions: carrier stream = 0.1 M $HClO_4$, detection potential = + 1V vs. Ag/AgCl/sat. KCl, flow rate = 4 ml $min^{-1}$, injection volume = 100 μl.

which carbon paste electrodes are subject to is minimised because of the continous passage of the carrier stream which cleans the electrode surface. This, in turn, saves time because electrode pretreatment stages can be omitted.

In Fig. 3, 17 successive records corresponding to a naltrexone solution are presented. The precision, expressed in terms of the relative standard deviation was 1.5% for a concentration level of $10^{-5}$ M ($n = 17$).

### 3.2. Electrode calibration

Calibration plots were constructed using the optimised parameters: electrode potential + 1.0V vs. Ag/AgCl/sat. KCl, flow rate 4 ml $min^{-1}$. The standards were, as usual, injected in triplicate.

The relationship between the peak current and the concentration of analyte injected (Fig. 4) produced a linear calibration plot between $2 \times 10^{-8}$ and $10^{-5}$ M. Treatment of this data by the method of least-squares gave the equation:

$$i_p/nA = 1.16 \times 10^8\ C/M + 11.82, \quad r = 0.9992,$$

$$n = 12$$

The detection limit (three times the standard deviation of the estimate [16]) was about $2 \times 10^{-8}$ M. Blank signals are injections of 0.1 M $HClO_4$. They could be due to the dispersion of the sample of the carrier solution in the carrier. At high potentials the influence of the oxidation of the background may produce fluctuations in the signal.

### 3.3. Analytical application

The oxidation process has been used to determine naltrexone in pharmaceuticals.

Liquid preparations (Antaxone, Pharmazan, Zambon group) nominally containig $50.0 \pm 2.5$ mg of naltrexone hydrochloride were used. This pharmaceutical, prepared for oral ingestion, is presented as a 20 ml solution of naltrexone in purified water and ethanol. It contains, in addition, 1 mg of saccharine. Five bottles of the portion I-22 were analysed following the procedure described above. A total of twelve determinations was made and a calibration curve was used to quantitate the contents.

The results compared favourably with those kindly offered by Pharmazan laboratories and obtained by HPLC of ion pairs on a column $C_{18}$ (5 μm), using heptansulphonate to form the ion pairs, a mobile phase of water, acetonitrile and tetrahydrofurane (80:13:7) and UV detection at

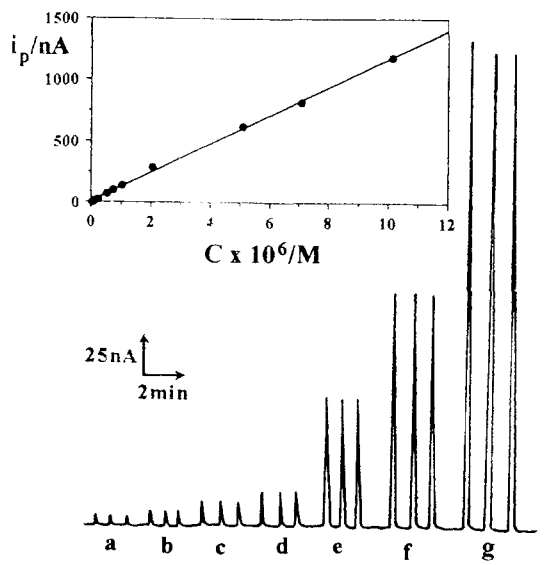



Fig. 4. Effect of naltrexone concentration on the FIA signal. Conditions: (a) blank injection; (b) $2 \times 10^{-8}$; (c) $5 \times 10^{-8}$; (d) $10^{-7}$; (e) $5 \times 10^{-7}$; (f) $10^{-6}$; and (g) $2 \times 10^{-6}$ M. Other conditions as in Fig. 3.

230 nm. The quantitation was carried out using an internal standard.

The average content of naltrexone obtained by voltammetry was 50.1 ± 1.5 mg, being 51.0 mg the mean value found by HPLC-UV. A *t*-test was carried out to evaluate the absence of systematic errors comparing both mean values and taking 51.0 as the true one. Considering a level of confidence of 95% and 11 degrees of freedom it can be concluded that there are no systematic errors in this methodology.

Although selectivity is inferior to that achieved by the voltammetric detection, results are adequate for this type of analysis since the matrix does not interfere, as can be observed in the voltammograms of the real samples recorded. Other amines such as cocaine, heroin and/or methadone are electroinactive at this pH value. Cocaine process is observable only at pH values higher than 6.5 [8], heroin gives rise to a single peak above pH 3 [7] and below pH 5 the oxidation process of methadone is not observed [6].

## 4. Conclusions

Flow injection analysis with amperometric detection is a valuable technique for the determination of naltrexone with good accuracy and precision. The electrochemical response shows a linearity with concentration over a broad range ($2 \times 10^{-8}$–$10^{-5}$ M). The proposed method is fast and cheap and was successfully applied to the quantitation in pharmaceuticals. It compared favourably with liquid chromatography with UV detection.

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