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Electrochemical studies and square wave adsorptive stripping voltammetry of the antidepressant fluoxetine

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Abstract

The electrochemical reduction of the antidepressant drug fluoxetine was investigated by cyclic, linear sweep, differential pulse and square wave voltammetry using a hanging mercury drop electrode in alkaline buffer solution in water and in a water/acetonitrile mixed solvent. Cyclic voltammograms in aqueous solution showed very strong adsorption of fluoxetine on the electrode with formation of a compact film. The effect of addition of different percentages of acetonitrile on the voltammetric response was evaluated. It is shown that acetonitrile protects the electrode surface, thus preventing the adsorption of fluoxetine as a compact film, although reduction occurs at more negative potentials. Adsorption was used to accumulate the drug onto the electrode surface. The adsorbed species were measured voltammetrically by reduction at -1.3 V in an aqueous 0.05 M Ringer buffer, pH 12, 20% acetonitrile v/v. Linear calibration graphs were obtained in the range 0.52 – 5.2 M. The quantification of fluoxetine in pharmacological formulations existing in the market was performed using adsorptive square wave cathodic stripping voltammetry, and compared with data from UV spectrophotometry. The method is simple and not time-consuming. A comparative high performance liquid chromatography assay with UV detection was performed. Recovery data for both methods are reported. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Fluoxetine; Electrochemistry; Antidepressant; Pharmaceutical formulations

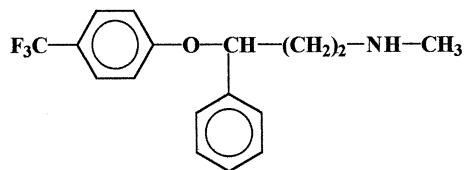
1. Introduction

The continuous demand for anti-depressive drugs with minimal side effects, mainly cardiovascular accidents or anticholinergic activity, gave rise to the development of the anti-depressant

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drug fluoxetine, *N*-methyl-8-14-(trifluoromethyl) phenoxybenzenepropanamine.



It works by inhibiting the uptake of serotonin by the neurons in the brain, enhances serotonin neurotransmission and has the longest half-life of all the selective serotonin reuptake inhibitors (SSRIs). It has been used for the treatment of major depression, obsessive–compulsive disorder, borderline personality and panic disorders, nervous anorexia and bulimia, autism, obesity, alcoholism, geriatrics, and deintoxication by cocaine [1–5]. Fluoxetine is clinically administered orally in the form of chlorhydrates and is the most widely prescribed antidepressant in the USA. The precise mechanism of action is not clear but it has less sedative, anticholinergic and cardiovascular effects than the tricyclic antidepressant drugs. Fluoxetine is metabolized to norfluoxetine which is also active. It is highly protein-bound and readily crosses the blood–brain barrier and the placenta, consequently also appearing distributed into breast milk. Carcinogenic studies provided evidence that fluoxetine is neither a complete carcinogen nor a tumor promoter [6].

The control of fluoxetine and norfluoxetine has been accomplished in blood serum using gas chromatography [7–12] and, to a larger extent, by high pressure liquid chromatography (HPLC) with fluorescence or UV detection [13–24].

This paper is concerned with the study of the adsorptive voltammetric behaviour of fluoxetine using a hanging mercury drop electrode (HMDE) in different buffer solutions. In the literature, no references were found concerning the use of electroanalytical techniques for the determination of this substance or studies of the electron transfer mechanisms of fluoxetine. Based on the results obtained, a square wave adsorptive voltammetric quantification method was developed. This procedure was applied to the determination of fluoxetine in commercial preparations existing in the market and the results were compared with those

obtained by the same determination using HPLC with UV detection.

2. Experimental

Fluoxetine chlorhydrate was kindly supplied by the laboratory El Lilly Pharmaceuticals (Indianapolis, IN, USA). All the chemicals used were of reagent grade quality and they were employed without further purification. The most conclusive experiments were performed in 0.05 M Ringer buffer ($\text{HPO}_4^{2-}/\text{PO}_4^{3-}$) in the range pH 9–12, and were prepared using purified water from a Millipore Milli-Q system.

The working electrode was a Metrohm multi-mode HMDE, the counter electrode a carbon rod and the reference electrode was AgCl/Ag/3 M KCl, used in a one-compartment cell of a 663 VA stand Metrohm. Voltammograms were recorded using a Autolab PSTAT 10 potentiostat/galvanostat running with model GPES version 3 software, from Eco-Chemie, Netherlands. The potential range studied was from -0.8 to -1.8 V vs. Ag/AgCl, cyclic voltammetry scan rates varying from 20 to 800 mV s^{-1} . Differential pulse voltammetry conditions were: pulse amplitude, 40 mV; pulse width, 70 ms; scan rate, 6 mV s^{-1} ; and square wave voltammetry conditions were: pulse amplitude, 40 mV; frequency, 50 Hz; potential step, 6 mV.

The HPLC system used was a Sykan model A 1210 liquid chromatograph, equipped with a model 3200 UV/Vis detector and connected to a computing integrator model PRIME version 2.2.6 chromatography data station. For chromatographic separation, a Technopak 10 C_{18} column (250×4 mm, 5 μM particle size) was employed. The separation was carried out at room temperature using, as the mobile phase, 40% acetonitrile:60% 0.05 M potassium dihydrogenphosphate (pH 4.7) filtered through a 0.45 μm filter and degassed with a helium sparge. A Hamilton 50 μl syringe was used for sample injection.

The pharmaceutical samples were prepared by mixing and the content of 10 capsules followed by weighing exactly around one-tenth of it. To this powder aliquot was added 80 ml of water in a 100

ml dilution flask. This was placed during 15 min in an ultrasonic bath, before completing the volume up to 100 ml with water. This procedure was repeated 10 times.

Adsorptive voltammetry measurements were carried out in the square wave voltammetry mode (SWV) under the following optimized conditions: accumulation potential, -0.8 V and accumulation time, 5 s, with stirring at 500 rpm. An equilibrium time of 5 s was allowed to elapse between the end of the stirring and the start of the potential scan from -1.0 to -1.8 V. The adsorptive cycle was repeated three times using a new mercury drop each time, the result being expressed as the average of the four measurements. Quantification of fluoxetine was performed by means of the standard addition method.

To calculate the recovery, another aliquot of the powder sample was weighed and a known number of milligrams of fluoxetine clorhydrate were added; the quantification procedure already described was carried out.

3. Results and discussion

The electrochemical reduction process of fluoxetine at the HMDE leads to very strong adsorption on the electrode surface, as seen from the cyclic voltammograms in Fig. 1, at pH 12. The shape of the cyclic voltammograms suggests that, in the conditions used, they correspond to a

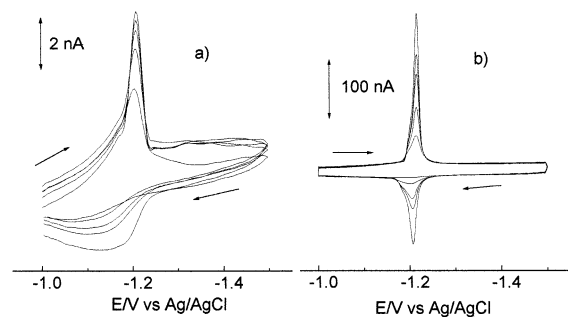


Fig. 1. Cyclic voltammograms of fluoxetine at scan rates of: (a) 90 mV s^{-1} ; (b) 600 mV s^{-1} . Concentrations: 6.36, 7.93, 9.50, 11.1 and $12.6 \mu\text{M}$ in 0.05 M Ringer buffer, pH 12, $t_{\text{dep}} = 5 \text{ s}$ at $E_{\text{dep}} = -0.8 \text{ V}$.

quasi-reversible system for adsorbed species, since there is only a very little separation of 10 mV between anodic and cathodic peaks. The curve is almost symmetric round E_p , the width at half-height, $W_{1/2}$, decreases slightly as the concentration increases, and the peak current is proportional to the scan rate, ν , for both the anodic and the cathodic peaks. Also the peak potential does not vary with scan rate, ν , and the oxidation peak only appears for very high scan rates (Fig. 1b). However, the adsorption peak height for the reduction does not vary linearly with concentration, suggesting blocking by formation of a compact film on the electrode surface [29–32].

The strong adsorption process taking place on the electrode surface corresponding to the accumulation of fluoxetine was confirmed by repetitive cyclic voltammograms recorded after dipping the HMDE in a stirred solution of the drug for a period of 5 s at -0.8 V. The short accumulation time gives substantial enhancement of the cathodic peak (first scan) compared with those of non-accumulated species (subsequent scans), thus indicating a rapid desorption of fluoxetine from the electrode surface.

The study of peak potential, E_p , versus pH, in Britton Robinson buffer, showed that the reduction signal for fluoxetine appears only for pH values higher than 8.5. This is because reduction only occurs at very negative potentials, as is predicted for this type of compound [24,25]. Consequently, the reduction peak can only be expected to be observed when very high pH value supporting electrolytes are used because in those experimental conditions, the negative potential range for HMDE is increased up to -2.0 V vs. Ag/AgCl, and the reduction peak can be recorded. Fluoxetine molecules are potentially basic on account of the unshared electron pair of the oxygen atom and at these pH values it is the unprotonated form that is reacting (Fig. 2). The peak potential was only shifted slightly to more positive values as the pH was increased, the slope corresponds to 6.1 mV per unit of pH. Thus the reduction of fluoxetine is pH independent.

The reduction reaction mechanism of fluoxetine is quite general for aromatic compounds [25–28]

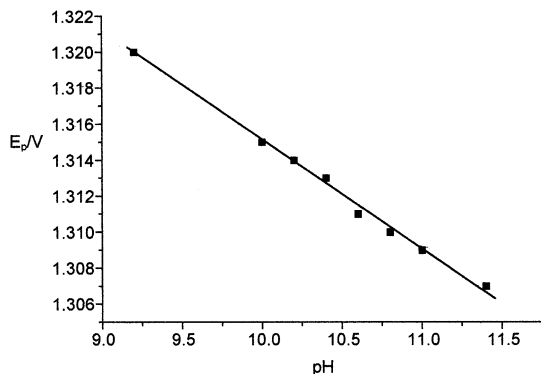


Fig. 2. Plot of E_p vs. pH for a 6.36 M fluoxetine solution in 0.05 M Ringer buffer solutions. The line corresponds to a slope of 6.1 mV per unit of pH.

and is considered to require the formation of a radical anion formed by electron transfer into an antibonding orbital of the arene linked to the oxygen. The peak current increased until pH 10.5 and then became constant until pH 12.5. Since the best definition of peaks was observed for pH 12 in Ringer buffer, this supporting electrolyte was chosen for subsequent experiments.

The strong adsorption process of fluoxetine on the electrode surface depended on the accumulation time and was investigated further. Without accumulation, when the concentration was lowered to 1 μM , a negligible current was observed. However, a well-defined peak was observed if a 60 s accumulation period preceded the potential scan. For micromolar concentrations, besides the adsorption peak, a diffusion peak, due to the reduction of diffusing molecules, appears for a long deposition time. Fig. 3 shows the diffusion peak as a shoulder before the adsorption peak in the negative potential scan [29]. A deposition potential of -0.8 V was chosen and accumulation times varying from 5 to 80 s were evaluated, after which CVs were recorded. For concentrations less than 1 M, 20 s deposition time led to the maximum current. However, at higher concentrations, the shape and peak height, in reduction as in oxidation, shows the same behaviour as described elsewhere [29–32] for the case of strong adsorption with formation of a compact film on the electrode surface. The peak potential was

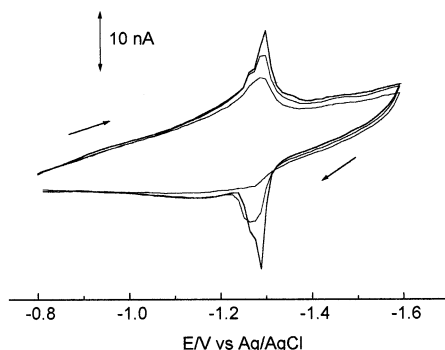


Fig. 3. Cyclic voltammograms of fluoxetine at 600 mV s^{-1} . Concentrations: 1.46, 2.04 and $2.62 \mu\text{M}$ in 0.05 M Ringer buffer, pH 12, $t_{\text{dep}} = 60 \text{ s}$ at $E_{\text{dep}} = -0.8 \text{ V}$.

shifted to more negative values and $W_{1/2}$ decreased as the concentration or the accumulation time was increased.

Quantitative determinations of fluoxetine were very irreproducible due to the very strong adsorption causing decrease of peak height in successive scans. When a buffer supporting electrolyte in an acetonitrile/water mixed solvent was used, the reproducibility of the peaks and the resolution of the peaks improved although the peak potentials were shifted ~ 200 mV to more negative values. Comparison between Fig. 4a and Fig. 4b shows the effect of using 20% acetonitrile/80% water as solvent.

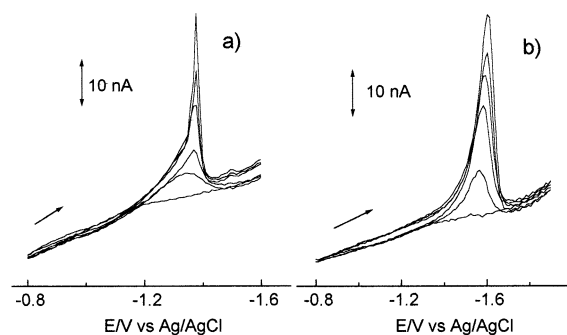


Fig. 4. Adsorptive linear sweep voltammetry of fluoxetine, (a) 0.05 M Ringer buffer, pH 12; (b) 0.05 M Ringer buffer, pH 12, 20% acetonitrile v/v. Scan rate, 800 mV s^{-1} ; concentrations: 0.92, 1.85, 2.76, 3.68 and $4.59 \mu\text{M}$, $t_{\text{dep}} = 20 \text{ s}$ at $E_{\text{dep}} = -0.8 \text{ V}$.

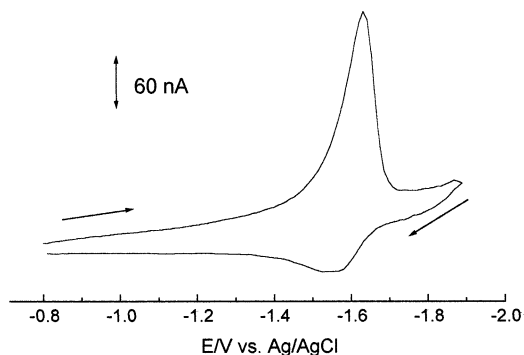


Fig. 5. Cyclic voltammogram of 12.5 μM fluoxetine in 0.05 M Ringer buffer, pH 12, 20% acetonitrile v/v. Scan rate, 800 mV s^{-1} ; $t_{\text{dep}} = 20$ s at $E_{\text{dep}} = -0.8$ V.

According to Ref. [33], the differential capacity of the electric double layer is a very sensitive function of the adsorption of organic molecules at the mercury electrode surface. At high positive and negative surface charges, the adsorption–desorption peaks on the C vs. ϕ curves, are very much lower or vanish entirely in the case of solution in organic solvents in comparison with aqueous solutions. The influence of the mixture of aqueous with organic electrolyte in the height and position of the peaks on the C vs. ϕ curves is caused by the salting-out of the organic substance [34], giving rise to a shift of the cathodic peak in the negative direction and an increase of the peak height. This is in good agreement with our experiments where the shift of the peak potentials ~ 200 mV to more negative potentials was observed.

The influence of different percentages of acetonitrile in the solvent was evaluated and it was found that 0.05 M Ringer buffer, 20% acetonitrile v/v, led to the best improvement of peak definition and height (Fig. 5). The cyclic voltammogram after accumulation of fluoxetine at -0.8 V shows that acetonitrile protects the electrode surface by preventing irreversible adsorption of fluoxetine.

In the presence of acetonitrile, the symmetry of the differential pulse voltammetric peak also improves and, since the resolution is better, it is possible to determine lower concentrations of fluoxetine down to 3.2×10^{-7} M. In these condi-

tions, the non-linear relationship between peak current, i_p , and $v^{1/2}$ shows again that the reduction process is not only diffusion controlled, but there is an important adsorption component.

In the presence of acetonitrile, different potential scan modes, i.e. linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV), were applied to the stripping analysis of the adsorbed fluoxetine (Fig. 6). It was found that the use of pulse techniques improves the sensitivity, as expected. The largest slope value in the plot of peak current vs. concentration, 14.3 nA M^{-1} , was obtained using SWV while values of 6.18 and 0.93 nA M^{-1} were achieved using LSV and DPV respectively. Therefore, square wave voltammetry was chosen for further work since this technique is less time consuming and shows the best peak resolution.

For electroanalytical purposes, the optimised conditions for square wave adsorptive stripping voltammetry found were accumulation of fluoxetine on the electrode surface during a total of 10 s at a potential of -0.8 V, 5 s with stirring at 500 rpm, followed by 5 s without stirring, supporting electrolyte 0.05 M Ringer buffer, 20% of acetonitrile v/v, frequency 50 Hz, pulse amplitude 40 mV, potential step 6 mV, and scan from -1.0 to -1.6 V.

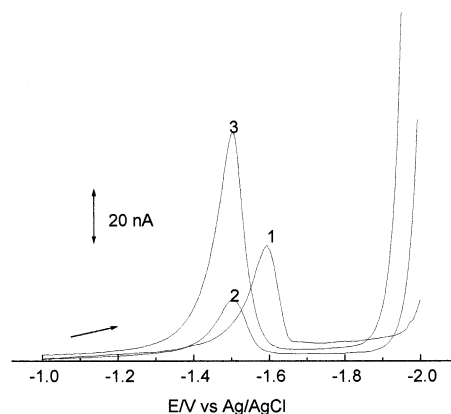


Fig. 6. Adsorptive stripping voltammograms of a 5.19 μM fluoxetine solution in 0.05 M Ringer buffer, pH 12, 20% acetonitrile v/v, $t_{\text{dep}} = 5$ s at $E_{\text{dep}} = -0.8$ V: (1) linear sweep voltammetry, scan rate 800 mV s^{-1} , (2) differential pulse voltammetry, scan rate 6 mV s^{-1} , (3) square wave voltammetry, frequency 50 Hz and potential step 6 mV.

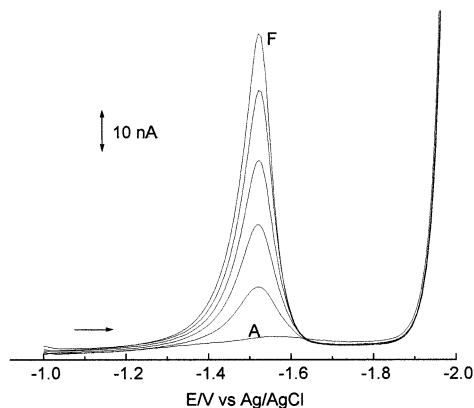


Fig. 7. Square wave adsorptive stripping voltammograms obtained after increasing the fluoxetine concentration in $1.05 \mu\text{M}$ steps from 0 (A) to $5.19 \mu\text{M}$ (F), $t_{\text{dep}} = 5 \text{ s}$ at $E_{\text{dep}} = -0.8 \text{ V}$.

In these conditions, a value of $3.9 \times 10^{-8} \text{ M}$ was determined for the detection limit (defined as three times the noise). The dependence of peak current on fluoxetine concentration was found to be linear over a range from 0.52 to $5.2 \mu\text{M}$. Fig. 7 shows square wave voltammograms obtained after successive standard additions of fluoxetine chlorhydrate, each addition corresponding to a $1.05 \mu\text{M}$ increase in concentration. A least-square treatment of the data in Fig. 7 yields a slope of 14.3 nA M^{-1} and an intercept of 0.42 nA , with a correlation coefficient of 0.9998 . Precision was calculated by 10 successive measurements of a $5.19 \mu\text{M}$ fluoxetine solution (accumulation for 5 s at -0.8 V) with a relative standard deviation of 2.8% .

The fluoxetine content of commercially available capsules, prepared as described in Section 2,

was determined directly using adsorptive linear sweep square wave voltammetry method, using the standard addition method. The results obtained by the electroanalytical method were in good agreement with those obtained by HPLC with UV detection using the same samples (Table 1).

4. Conclusions

The reduction of fluoxetine is pH independent and occurs at very high potentials, which means that it can be studied only at pH values higher than 8.5 . The use of buffer electrolyte in a mixed acetonitrile/water solvent proved very convenient for preventing strong adsorption of the analyte on the electrode surface and enabling better reproducibility and sensitivity. Adsorptive linear sweep square wave voltammetry permitted accurate quantification of fluoxetine in commonly used pharmaceutical drugs in the micromolar range after a very simple and rapid sample treatment. Good precision was obtained (relative standard deviation = 2.8% ; $n = 10$). This electroanalytical method can be used for determination of therapeutic doses of fluoxetine in biological fluids if coupled with high performance liquid chromatography (HPLC) with electrochemical detection.

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Table 1
Electrochemical quantification of fluoxetine in pharmacological formulations^a

Sample	SWV			HPLC		
	mg/cap	RSD (%)	Recovery (%)	mg/cap	RSD (%)	Recovery (%)
A	20.0	3	100	23.0	6	112
B	19.6	0.3	101	19.0	4	116
C	20.0	3	98	20.0	7	120
D	20.0	3	102	20.0	2	100

^a All formulations refer to 20 mg of fluoxetine per capsule. RSD (%), relative standard deviation.

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