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SIMPLE AND FAST SQUARE WAVE VOLTAMMETRIC METHOD FOR HISTAMINE H2-RECEPTOR ANTAGONIST FAMOTIDINE QUANTIFICATION

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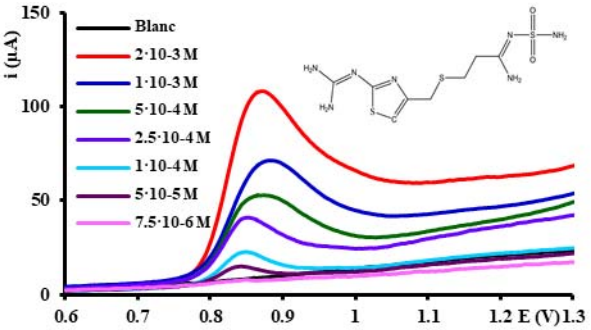
In the present work cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for electrochemical studies of famotidine on an unmodified pencil graphite electrode. It was established that the electrode process depends on the pH and it is controlled by diffusion. Under optimized conditions (0.5 M potassium hydrogen phthalate buffer KHP pH 4.6), linearity was achieved on two concentration ranges ($5 \cdot 10^{-6}$ M – $1.8 \cdot 10^{-4}$ M and $1.8 \cdot 10^{-4}$ M – $2 \cdot 10^{-3}$ M), detection and quantification limits being calculated as $1.29 \cdot 10^{-6}$ M and $3.92 \cdot 10^{-6}$ M, respectively. The developed SWV method was applied with good results for the rapid, affordable, easy and reliable analysis of pharmaceutical samples.



INTRODUCTION

Famotidine (FMT), 3-[2-(diaminomethyl-eneamino)thiazol-4-ylmethylthio]-N-sulfamoyl-propionamidine, is an active pharmaceutical ingredient commonly used to cure gastric and duodenal ulcers, as well as to prevent their recurrence.[1] FMT is a histamine H2-receptor antagonist and it is also employed in the treatment of the gastroesophageal reflux disease.[2] This disease and its major symptoms affect roughly 40 up to 85% of the pregnant women.[3] FMT can be administrated either intravenously as an infusion or orally and it is not completely absorbed in the gastrointestinal tract. A small portion of FMT is metabolized in liver, but the most of it is found unchanged in urine. Due to the fact that the therapeutic concentration is rather low (40 mg/day), there is a small plasmatic concentration (20–150 ng·mL^{-1}).[4]

Taking into consideration that FMT proved to be more forceful than cimetidine and ranitidine (other well-known histamine receptor antagonists), having also fewer consequences on hemodynamics, it acquired a larger clinical use.[3] Regarding the quantitative determination of FMT, the British Pharmacopoeia recommends a method based on thin-layer chromatography, while the US Pharmacopeia suggests a potentiometric method in non-aqueous medium and a high performance liquid chromatography (HPLC) method with UV detection.[5]

In the literature there were reported various FMT quantification methods, based on several techniques such as: HPLC, high performance thin-layer chromatography, capillary electrophoresis, UV molecular absorption spectrometry, spectrofluorimetry.[6] Some of these methods need the use of complex and expensive equipment and imply tedious sample preparation steps, making the analysis more

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complex. Therefore, new simple, rapid and cost-effective analytical methods that allow selective and accurate FMT determination from various pharmaceutical and biological samples are still required.

Electroanalytical methods possess certain advantages: simplicity in the preparation of samples, short analysis time, use of fewer reagents and simpler and cheaper equipment, therefore they represent a convenient alternative in the cost-efficient analysis of pharmaceutical samples. One disadvantage of most of the electrochemical methods is represented by the necessity of an additional step which consists in the regeneration of the electrode surface. But even this flaw can be overcome by using the disposable pencil graphite electrode (PGE). There are few reports in the literature regarding the FMT electrochemical quantification using several types of working electrodes from pharmaceutical products and biological samples.[7-13]

The purpose of this study was to establish the electrochemical behaviour of FMT on unmodified and non-activated PGE and to develop a simple, rapid and cheap square wave voltammetric method for FMT determination in pharmaceutical samples.

RESULTS AND DISCUSSION

The selection of the working electrode

The electrode material has an important effect on the electrochemical behaviour of the analyte, so initially four working electrodes were considered: PGE, glassy carbon electrode (GCE), platinum electrode (Pt) and gold electrode (Au). The PGE consisted of 0.5 mm HB Rotring pencil leads, that contain graphite and clay in the same ratio and it is well known for generating high voltammetric signals and low background noises.[14] The surface areas of the working electrodes were: 15.896 mm^2 for PGE, 7.065 mm^2 for GCE and 3.140 mm^2 for Au and Pt electrodes, respectively. Cyclic and square-wave voltammograms were registered on each of the four electrodes, for a solution of 10^{-4} M FMT in 0.5 M KHP, pH 4.60. Each of the cyclic voltammograms showed a single anodic peak, regardless of the used electrode, but the best shaped voltammogram and the highest signal were obtained on PGE. The results achieved by using cyclic voltammetry (CV) and square wave voltammetry (SWV) pointed out that there was a direct correlation between the peak current and the geometric electroactive surface area of the working electrode. For a better characterization of the electrode material, the sensitivities ($A/cm^2 \cdot M$) of the working electrodes were compared and PGE proved to possess the highest sensitivity (Figure 1). Moreover, when employing PGE, less positive values of the peak potentials were obtained. Therefore, PGE was employed as working electrode for all subsequent investigations.

The influence of the supporting electrolyte pH

Cyclic and square-wave voltammograms were recorded on PGE for a solution of 10^{-4} M FMT using Britton Robinson buffer (BRB) as supporting electrolyte (pH 1.81 – 11.92) (Figure 2), in all cases an irreversible oxidation peak being observed. A shift of the peak potential to less positive values was observed when the pH value increased, fact that demonstrates that in the electrode process protons are also involved.

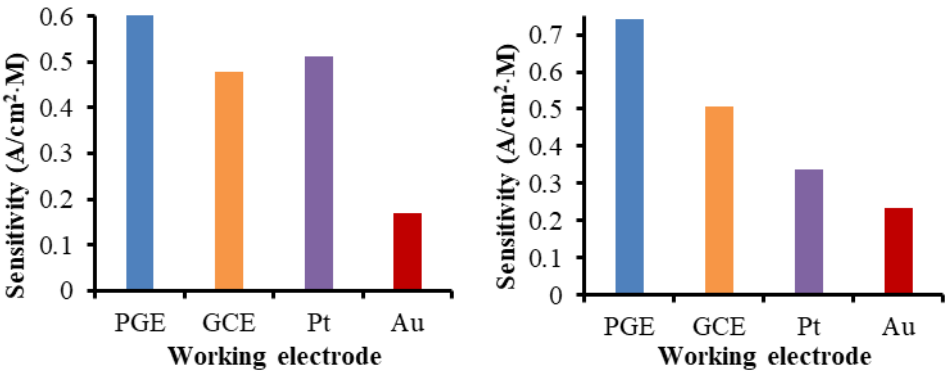

Fig. 1 – Sensitivities for FMT determination in 0.5 M KHP pH 4.60 on different electrodes: CV (a); SWV (b).

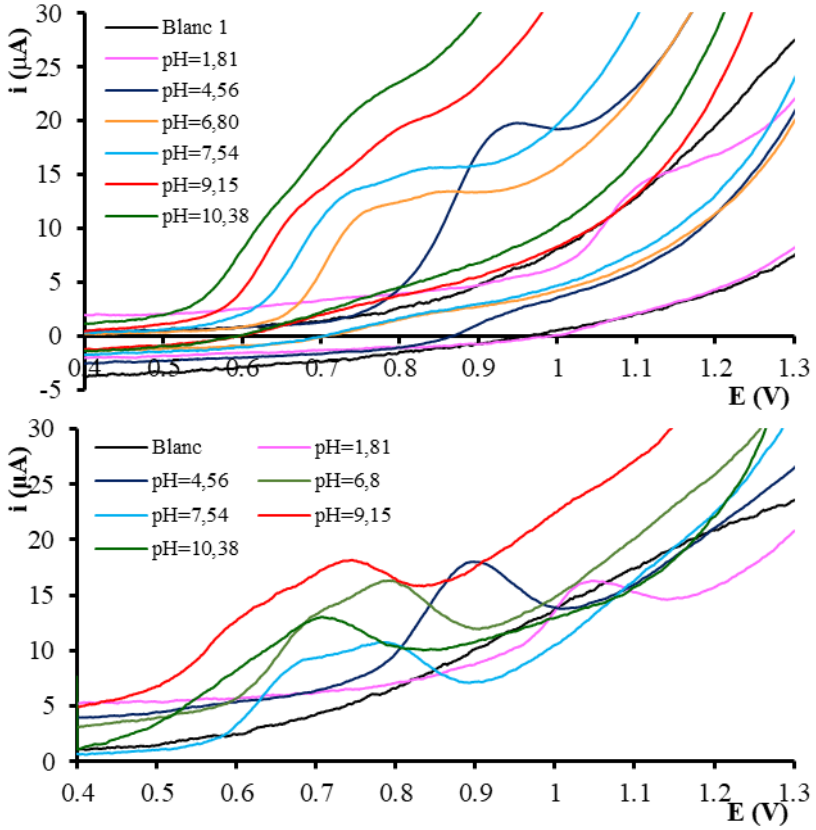

Fig. 2 – Cyclic (a) and square-wave (b) voltammograms for 10^{-4} M FMT in BRB (pH 1.81 – 11.92).

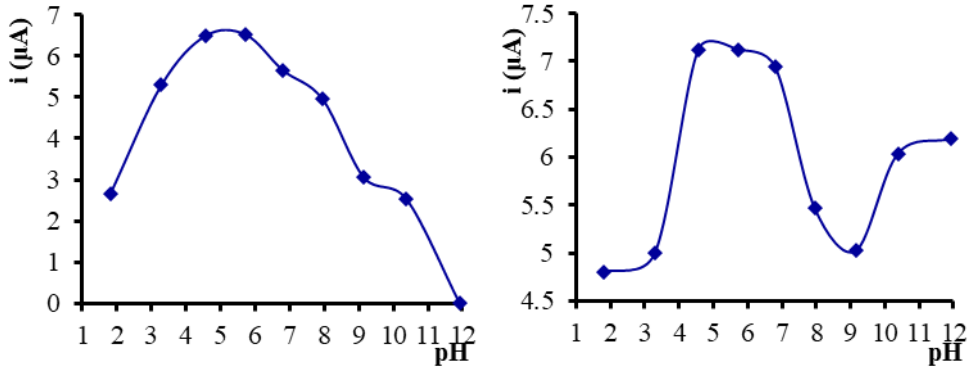

Fig. 3 – i = f (pH) graphical representations for 10^{-4} M FMT in BRB (pH 1.81 – 11.92): CV (a) and SWV (b).

The peak potential varied linearly with the pH value, but close to pH 7.50, a change of the slope was observed, probably due to the protonation of the guanidine group of FMT in acidic medium.[11] The equations of the E_p = f (pH) dependencies were: E_p = -0.0587pH + 1.1893 (pH 1.81-7.50) and E_p = -0.0264 pH + 0.9349 (pH 7.50-11.92) for CV and: E_p = -0.0541pH + 1.1336 (pH 1.81-7.50) and E_p = -0.026pH + 0.9243 (pH 7.50-11.92) for SWV. At pH values lower than 7.50, the slopes of the equations are close to the theoretical value of 0.059*x*/*n* (V/pH), where *x* is the number of protons and *n* the number of electrons transferred in the electrochemical reaction, meaning that the number of electrons and the number of protons are equal. At pH values higher than 7.50 the slopes of the equations are closer to half of the theoretical one, hence the electron to proton ratio is 2.

The graphical representation of the peak currents dependence on the pH value lead to the conclusion that the peak current is also influenced by the pH. The best shaped cyclic and square wave voltammograms along with the highest signals were obtained for FMT in BRB solutions with pH between 4 and 7 (Figure 3).

Taking into consideration all the above, in all future studies supporting electrolytes with pH values between 4 and 7 were used.

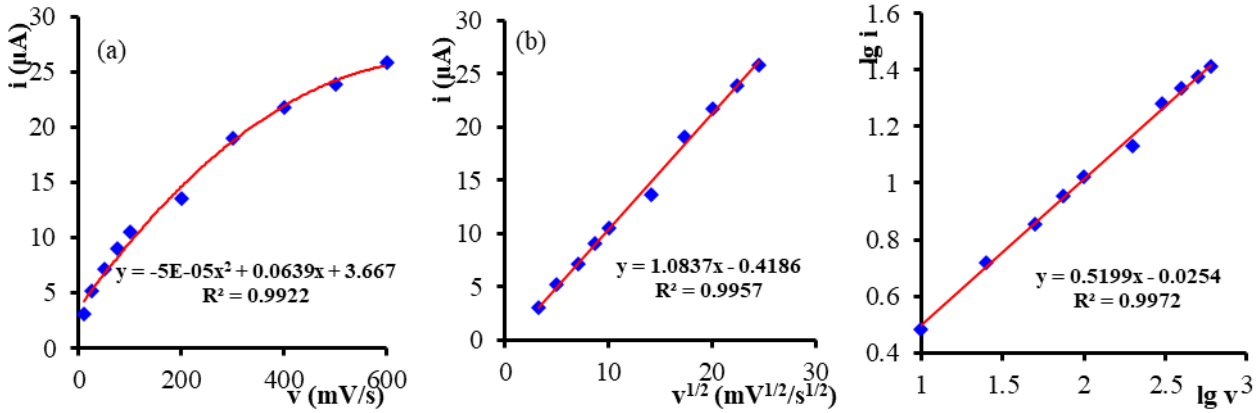

Fig. 4 – Dependencies (a) i = f(v), (b) i = f($v^{1/2}$), (c) lg i = f(lg v) characterizing the cyclic voltammograms obtained for 10^{-4} M FMT in 0.5 M KHP pH 4.60.

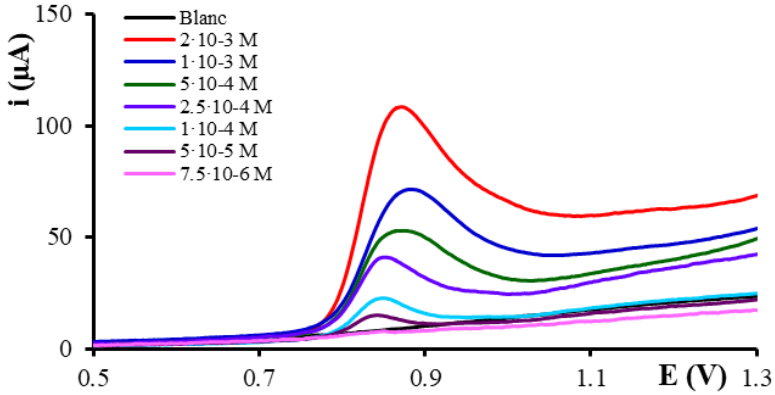

Fig. 5 – Square-wave voltammograms for FMT solutions with different concentrations.

The influence of the nature and concentration of the supporting electrolyte

The voltammetric response is affected by the nature of the supporting electrolyte. This is the reason for which the influence of various supporting electrolytes (potassium hydrogen phthalate buffer (KHP), phosphate citric acid buffer (PCAB), acetate buffer solution (ABS), phosphate buffer solution (PBS), BRB) on the oxidation peaks was studied employing both CV and SWV. Cyclic and square wave voltammetric signals were obtained in all cases, but the best defined voltammograms (not shown) and the highest peak currents were recorded in KHP pH 4.60. Moreover, different concentrations of KHP pH 4.60 were tested (0.01 M; 0.05 M; 0.1 M; 0.2 M; 0.3 M; 0.4 M; 0.5 M). The best results were attained in 0.5 M KHP pH 4.60, this being chosen as the supporting electrolyte for all the following measurements.

The influence of the potential scan rate

The influence of the scan rate on the voltammetric behaviour of FMT was studied via CV in order to establish the nature of the electrode process. Cyclic voltammograms (not shown) were recorded for 10^{-4} M FMT in 0.5 M KHP pH 4.60, on PGE at different scan rates ranging between 10 mV/s and 600 mV/s. As shown in Figure 4, the plot i = f(v) is not linear, while the representation of i = f($v^{1/2}$) is linear, respecting the Randles-Sevcik equation. Furthermore, the slope of the equation that characterizes the lg i = f(lg v) dependence is close to the theoretical value of 0.5. All these criteria led to the conclusion that the FMT oxidation is a diffusion controlled electrode process.

Quantitative determination of FMT using SWV on PGE

The effect of the FMT concentration on the square wave voltammetric signal on PGE in 0.5 M KHP pH 4.60 was investigated within the concentration range $1{\cdot}10^{-7}$ M – $2{\cdot}10^{-3}$ M (Figure 5).

The intensity of the oxidation peak varied linearly with FMT concentration obtaining two linear ranges: $5{\cdot}10^{-6}$ M – $1.8{\cdot}10^{-4}$ M (i (A) = $0.1505{\cdot}C$ (M) – $1.0435{\cdot}10^{-7}$; R^2 = 0.9994) and $1.8{\cdot}10^{-4}$ M – $2{\cdot}10^{-3}$ M (i (A) = $0.0321{\cdot}C$ + $2.1414{\cdot}10^{-5}$; R^2 = 0.9992).

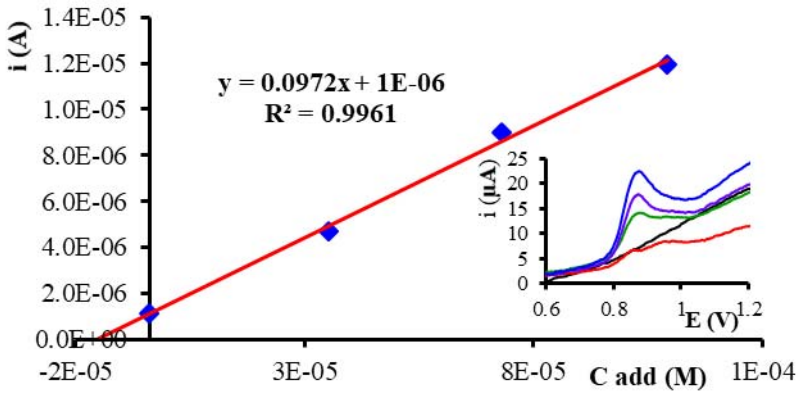

Fig. 6 – Square-wave voltammograms for Famotidine Zentiva 40 mg tablets solution in 0.5 M KHP pH 4.60 before and after each of the additions of $2 \cdot 10^{-3}$ M FMT standard solution.

The detection limit and quantification limit were calculated using the regression data of the lower linear range as $3.3 \cdot s_a/b = 1.29 \cdot 10^{-6}$ M and $10 \cdot s_a/b = 3.92 \cdot 10^{-6}$ M, where b is the calibration curve slope and s_a is the standard deviation of the intercept.

The repeatability of the PGE response was assessed by performing 10 SWV measurements at each of the 3 levels of FMT concentrations within the linearity range: $7.5 \cdot 10^{-6}$ M, $7.5 \cdot 10^{-5}$ M and $2 \cdot 10^{-3}$ M. For each measurement a new pencil graphite lead was used as working electrode (another PGE). The obtained RSD% values were: 6.70 %, 4.56 % and 1.82 %, respectively, all of them being within the approved limits depending on the concentration level.[15]

Analytical applications of the developed SWV method

The usefulness of the SWV method developed was verified on real samples: Famotidine Zentiva 40 mg tablets were analyzed.

The initial sample prepared according to the "Experimental" was diluted so that the sample concentration lie within the established linear concentration range and analyzed using the standard addition method. The square wave voltammograms recorded for the sample solutions showed just a well-defined anodic peak, representative for the FMT oxidation. This denotes the absence of interferences from the other components present in the analyzed pharmaceutical formulation. The peak current increased linearly with the added FMT concentration, thus allowing FMT quantitative determination (Figure 6).

The peak currents before and after each of the three additions were employed to calculate the FMT content from the analyzed tablets and the result was (39.58 ± 0.64) mg / tablet (RSD % = 1.61 %). The average percentage recovery value was (100.36 ± 0.64) %. The value of the relative percentage error (er % = 1.05 %) emphasizes a very small difference between the FMT content declared by the manufacturer of the pharmaceutical formulation and the one determined by SWV on PGE. Thus, using PGE, the achievement of comparable and reliable results is ensured by the proposed SWV method.

EXPERIMENTAL

Equipment and reagents

A Voltalab PST 050 Radiometer voltammetric assembly equipped with a potentiostat and a polarographic stand MDE 150 was used. The electrochemical cell consisted of a working electrode (PGE, GCE, Au or Pt), a reference electrode (Ag/AgCl, KCl (3 M)) and an auxiliary electrode (Pt). 0.5 mm HB Rotring pencil lead represented the PGE obtained as already described. [16] For each measurement a new PGE was used. For the pH measurements a pH/mV-meter InoLab WTW 730 equipped with a combined pH-glass electrode was employed.

A stock solution of FMT ($2 \cdot 10^{-3}$ M) was daily prepared by dissolving 0.0034 g FMT in bidistilled water and dilution to 5 mL and subjected to ultra-sonication for 30 minutes. Subsequently, intermediate solutions ($1 \cdot 10^{-3}$ M, $1 \cdot 10^{-4}$ M, $1 \cdot 10^{-5}$ M) were also prepared. Working solutions with lower concentrations were obtained by dilution with the appropriate supporting electrolyte: KHP (pH 4.60; 5.60), PCAB (pH 4.60; 5.60), ABS (pH 4.03; 5.00), PBS (pH 6.00; 6.50; 7.00) and BRB (pH 1.81 - 11.92).

Voltammetric measurements

The cyclic voltammograms of FMT were recorded from 0.4 to 1.3 V, with a scan rate of 100 mV/s, unless otherwise specified. SWV was applied within the same potential range using the following optimized instrumental parameters: pulse amplitude 50 mV, ramp step amplitude 1 mV, ramp step duration 0.02 mV.

Sample preparation

Commercially available pharmaceutical tablets (Famotidine Zentiva 40 mg), which contain only FMT and excipients, were analysed. Ten tablets were weighted (2.0121 g) and grounded to a fine powder. An amount of this powder equivalent to 0.0400 g FMT was weighted and transferred into a 100 mL volumetric flask, filled half way with bidistilled water and ultra-sonicated for 30 minutes. Afterwards, the volumetric flask was filled up to the mark with bidistilled water. The real sample solution was filtered and an aliquot of 0.1 mL was 100-fold diluted with 0.5 M KHP pH 4.60. From this solution, a volume of 10 mL was put in the voltammetric cell. The analyses were carried out on three replicate samples, using the standard addition method. Each time three measurements were made. Square-wave voltammograms were recorded before and after each of three successive additions of 0.2 mL of $2 \cdot 10^{-3}$ M FMT stock solution.

CONCLUSIONS

The electrochemical behavior of FMT on PGE was investigated by CV and SWV and, based on the obtained results, a SWV method was developed for FMT quantification from commercially available pharmaceutical formulations. Although the SWV on PGE proposed method does not have a very low detection limit, this aspect is less important when the method is applied to the quantitative determination of FMT in different pharmaceutical preparations. However, the voltammetric method described for FMT analysis has two linear ranges covering over three orders of magnitude, the most important feature being related to the fact that it uses an user- and ecofriendly, unmodified, disposable working electrode, which translates into a faster and cheaper method of analysis. The SWV method was successfully applied to the FMT quantitative determination in pharmaceutical tablets.

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