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Ultrasensitive Flow-Injection Electrochemical Method Using Fast Fourier Transform Square-Wave Voltammetry for Detection of Vitamin B_1

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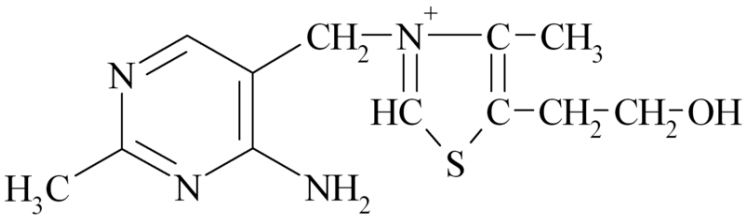
A simple and rapid fast Fourier transform square-wave voltammetry (FFT-SWV) method for the determination of Thiamine (vitamin B_1) using a platinum ultramicroelectrode (UME) in flow-injection system is proposed. The procedure is based on the changes in admittance voltammogram of the UME (in 0.1 mol/L potassium hydrogen phthalate) caused by adsorption of the Thiamine on the electrode surface. To obtain the much sensitivity the effective parameters such as frequency, amplitude, stripping potential and time were optimized. The best performance was obtained with the pH value of 3, pulse amplitude 60 mV, frequency 1200 Hz, accumulation potential of -300mV and accumulation time of 0.3 s. Furthermore, signal-to-noise ratio has significantly increased by application of discrete FFT method, background subtraction and current-time integration of the electrode response over a selected potential range. The calibration curve was linear over the range 5.0×10^{-10} to 4.0×10^{-7} mol/L for Thiamine. As a result, C_{DL} of 8.0×10^{-11} mol/L and LOQ of 5.6×10^{-10} mol/L were found for determination for Thiamine. The relative standard deviation at concentration 5.0×10^{-7} mol/L is 2.1% for five reported measurements and the optimized procedure in a flow injection analysis system applied with excellent results in the determination of Thiamine in commercial pharmaceutical preparations.

Keywords: Thiamine, fast Fourier transform square wave voltammetry, FFT voltammetry, flow injection analysis

1. INTRODUCTION

Thiamine, a water-soluble vitamin, is a natural nutrient present in many foods and is also added as an essential nutrient. Its chemical structure is shown in Scheme 1. It participates in the normal sugar metabolic process of body, maintains the normal function of nerve, heart and digestion, and it is mainly used to cure beriberi and varies of polyneuritis in clinical. Thiamine is very important to the brain, especially in terms of emotional health and well being, and also is useful for focus and concentration. Thiamine deficiency is frequently seen in alcoholics because heavy drinking limits the ability of the body to absorb this vitamin from foods [1-3]. Therefore, the determination of Vitamin B_1 is one of the important contents in food and clinical analysis. There were many methods for the determination of Thiamine, including the classical gravimetric [4] and titration methods [5], electrochemical analysis method (chemosensors and biosensors) [6, 7], thin-layer chromatography [8], gas chromatography [9], capillary electrophoresis [10], high performance liquid chromatography [11,12] and spectrophotometry [13], etc. Among them, the gravimetric method, titration and many electrochemical analysis methods were suitable for the determination of macro Vitamin B_1.



Scheme 1. Chemical structure of Thiamine

The determination of several analytes using flow injection analysis (FIA) has a number of potential advantages because of the flexibility of FIA assemblies and their typically high throughput, modest reagent consumption and adaptable sensitivity (dependent on the particular detector employed) [14].

Recently, our research team has focused on low level determination of some pharmaceutics by using different electrochemical and chemometrics methods [15-20]. In this paper a sensitive electrochemical method was introduced for determination of Thiamine. The SWV has recently been shown to be helpful for environmental detection of several compounds [21,22].

At this point a fundamentally different approach to SWV measurement is described, in which the detection limits are improved, while preserving the information content of the SW voltammogram. Here, application of FFT method with SWV was found enhance sensitivity of the electrochemical method for trace detection of various compounds [23-31]. The method is formulated to separate the voltammetric and background signal in frequency domain by using discrete fast FFT procedure, and

calculated total admittance of the UME electrode. The admittance response is measured by FFT-SWV while rapid alternating potentials are applied during a staircase scan. The method that introduced in this paper is very sensitive, inexpensive and fast for detection of Thiamine.

2. EXPERIMENTAL PART

2.1 Instrumentation

Voltammetry experiments were performed using a setup comprised of a PC PIV equipped with a data acquisition board (PCL-818H, Advantech Co.) was used to output an analog waveform to the working electrode and acquire current readings from the working electrode that connected to a custom made potentiostat. The diagram of electrochemical FIA cell is depicted in Fig. 1. The data acquisition board and accompanying dynamic link libraries allowed waveform generation and current sampling to be synchronized, which was essential in interpreting FFT-SWV response.

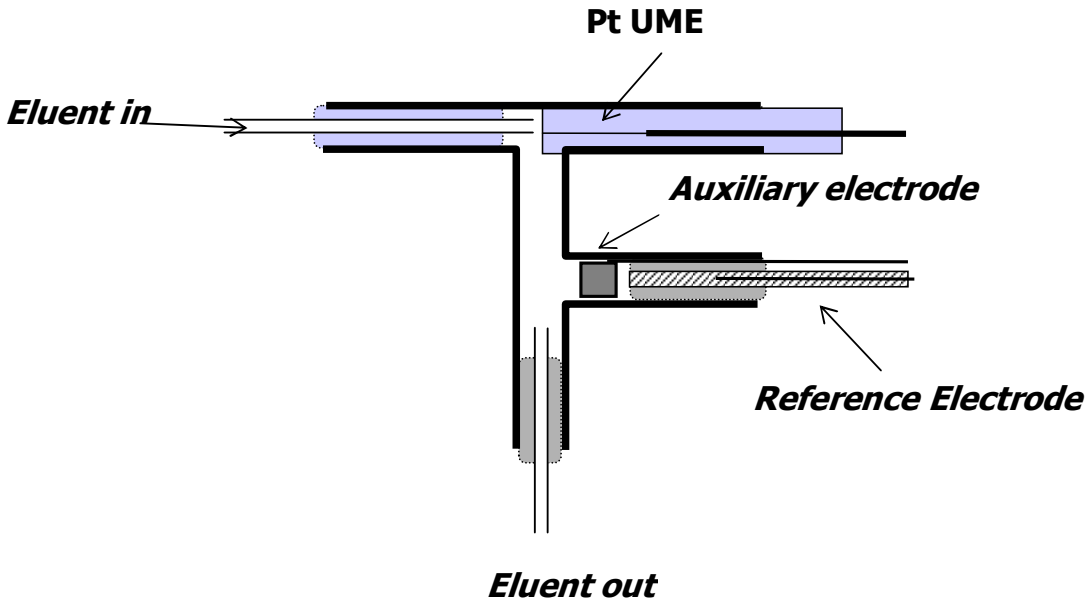


Figure 1. The diagram of electrochemical FIA cell

The potentiostat software was developed using Delphi 6.0 to repeatedly apply a waveform to the working electrode and precisely acquire, analyze, and store the data. The data could be interpreted in real time, or stored data could be loaded to generate voltammograms. The algorithms used to interpret the current response from each waveform cycle were discussed before [32]. Most of the waveform parameters could be modified from within the software; including the accumulation potential, accumulation time, SW frequency, amplitude, initial/final of potential scan, and the value of the experiment run time.

2.2. Materials and reagents

Vitamin B_1 was obtained from Asiamerica Ingredients, Inc. and was used without further purification. All other reagents were of analytical-reagent grade and used as received. Stock solutions were prepared daily by dissolving vitamin in buffer solution just before use and were used without removal of dissolved oxygen. Buffer solution of 0.1 mol/L potassium hydrogen phthalate (KHP) and 0.1 mol/L sodium chloride (pH 3) was used as supporting electrolyte solution [33]. A stock solution of Thiamine for electrochemical determination was prepared by dissolving 3 mg of it in supporting electrolyte solution in a 10 mL volumetric flask. In all experiments background electrolyte solution was made by double distilled water.

The equipment for FIA included a 10 roller peristaltic pump (Home made) and a four-ways injection valve (Supelco Rheodyne Model 5020) with a 60µL sample loop. In all experiments, described in this paper, the flow rate of eluent solution was 2.5 mL/min.

The Pt–UME was constructed from a 25µm diameter platinum wire (Goodfellow). In all measurements, an Ag (s) | AgCl (s) | KCl (aq, 1 mol/L) reference electrode was used. The auxiliary electrode was made of a Pt wire, 1 cm length and 0.5mm in diameter.

2.3. Electrochemical setup

As is shown in Fig. 2, the potential excitation waveform was programmed in a way to improve the sensitivity of the FFT-SWV technique. The potential waveform consisted of three sections; (a) electrode conditioning and (b) accumulation (c) measurement. The electrode conditioning section includes three potential steps, E_{c1} to E_{c2} (for cleaning the electrode surface from adsorbed compounds) and the potential step E_s (for accumulation of Thiamine).

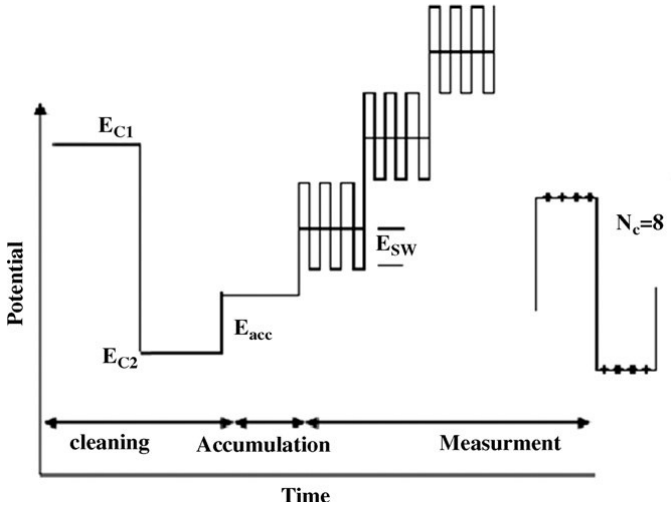


Figure 2. The diagram of the potential waveform used in measurements.

A typical experiment consisted of three consecutive steps with the following experimental conditions: The electrode was held at E_{c1} potential (1600mV) for 60ms, the E_{c2} potential at −200mV for 60ms, in which the electrode surface was strongly oxidized and reduced in order to remove adsorbed molecules. This section is followed by accumulation potential; E_s at −100mV for 500 ms. The measurement section of the waveform contains a multiple SW pulses with amplitude of E_{sw} and frequency of f_o, were superimposed on a staircase potential function, which was changed by a small potential step of ΔE. The values of E_{sw} (amplitude of SW) and ΔE were in a range of few mV (10 to 100 mV). In the section of potential wave form, the data current were sampled four times per each SW polarization cycle. After preparing the solution, the measurements were performed in the continuous FFT-SWV mode.

2.4. Sample preparation assay

Twenty tablets were weighed, finely powdered and portions equivalent to 100mg Thiamine were transferred into 100 mL volumetric flask; 50 mL distilled water was added, shaken thoroughly to dissolve, made up to volume and mixed well. Suitable aliquots of solution 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 50 µL of the resulting solution was added to a 100 mL volumetric flask and made up to volume with 0.1 mol/L KHP to yield starting concentration of 10.0 ng/ mL.

3. RESULTS AND DISCUSSION

The changes in admittance of the platinum electrode in 0.1 mol/L KHP into the eluent solution, caused by the injection of a solution of 60µL of 5.0×10^{-6} mol/L Thiamine is shown in Fig. 3. The FFT–SWV modulation had amplitude of 60 mV and a frequency of 1000 Hz. The peaks at the potentials 50-200- and 900-1200 mV at the voltammogram are due to hydrogen reduction and oxide layer formation on the surface of platinum electrode respectively (see Equation 1). When the electrode potential passes the zero charge potential changes in the voltammogram admittance is caused by the adsorption of hydrogen ion exchange at the Helmholtz layer. The second peak, with a shoulder, is related to oxidation of the electrode surface, as follows,

$$Pt + 2H_2O \rightarrow PtO_2 + 4H^+ + 4e^- \quad (1)$$

this reaction led to formation of oxide layer. The peak signal change during time after injection of analyte is shown in Fig. 3(a). A peak decline takes place in certain potential rang at the FFT–SWVs admittance, which is as a result of the analyte signal. Due to the inhibition of the electrode surface processes by the adsorbed Thiamine.

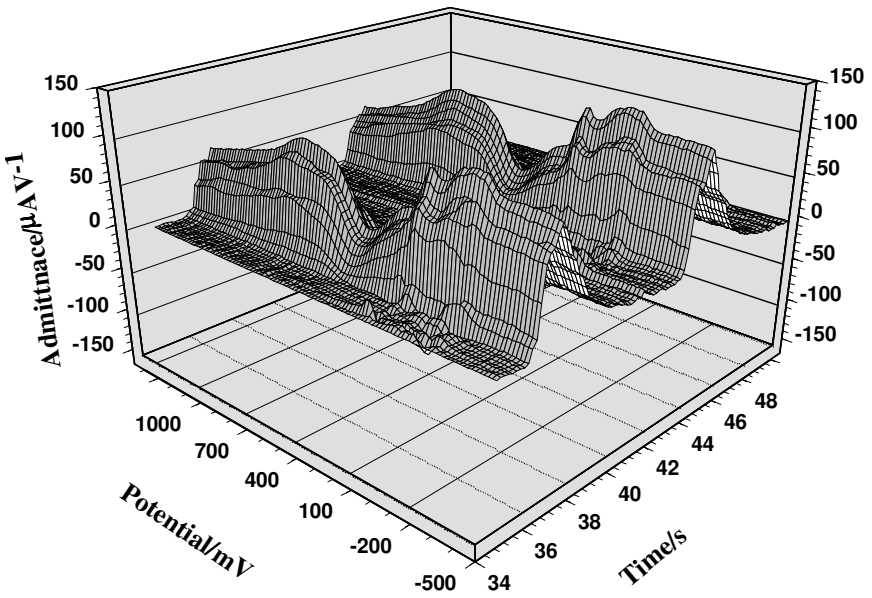
In Fig. 3(b) a differential form of the voltammograms is shown, where the average of first five voltammogram is stored in the computer memory and the subsequent voltammograms are subtracted from it based on the following equation.

$$\Delta A(s\tau) = A(s,E) - A(sr,E) \quad (2)$$

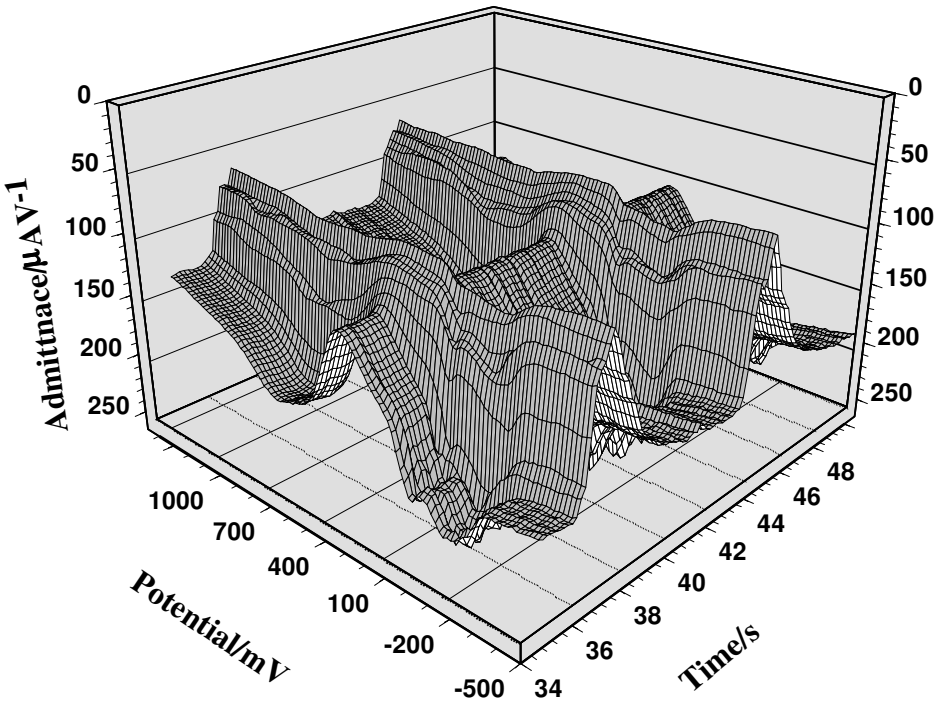
where, s is the sweep number, τ is the time period between subsequent sweeps, A (s, E) represents the admittance of the FFT–SW curve recorded during the *s*-th sweep and A (s_r, E) is the reference admittance of the FFT–SW curve. In this figure it can be also noted that the analyte signal extends over different potential ranges of the FFT–SWV.

Adsorption of organic molecules in solution on the surface of platinum can be an extremely complex process. This electrochemical reaction involves the desorption of adsorbed water molecule, hydroxyl, or electrolyte anions by adsorption of the analyte. Furthermore, adsorption of species on the electrode surface has been avoided in flowing solutions as it causes electrode fouling and analyte carryover. Such changes in the electrode surface concentrations and diffusion layer conductivity will result in a change in the charging current response.

When FFT–SWV is used to monitor a flowing system, analyte adsorption will lead to a measurable change in the admittance response. Moreover, faradic FFT–SWV current may be caused by chemisorptions of the analyte on the UME surface. Actually, this method would be very applicable for detection of electroactive and non-electroactive compounds, via the adsorption or redox reaction of substances) and coupling by chromatographic method make the system technique very effective to separate and determine the trace amount of compounds.



(a)



(b)

Figure 3(a) FFT square wave voltammograms at a 25 μm Pt UME recorded during a flow-injection experiment. The eluent was 0.1 mol/L KHP, the flow rate was 1 mL/min, and the frequency was 1000 Hz. The injected solution (50μL) contained 5.0×10^{-6} mol/L Thiamine in 0.1 mol/L KHP. (b) Graph (a), when the average of five Pt FFT SW voltammograms subtracted from the displayed voltammograms.

Using FFT–SWV technique, admittance response (in response to an alternating potential) can be examined to determine the time dependence of the change in the current response due to analyte adsorption. It should be noted that in this method both charging and faradic currents may potentially carry useful analytical information due to all processes studied involve adsorption of analytes. It is helpful in FFT–SWV to collect more current samples near the end of the forward and reverse pulses and use signal averaging to increase the ΔQ. In the traditional Osteryoung SWV method, the current is sampled at two points for each square wave, t_1 (the end of the first SW pulse) and t_2 (the end of the second SW pulse). In this technique, the majority of the charging current will have decayed at the end of each pulse, leading the faradic current to be sampled independently. The difference current [(current at t_2)−(current at t_1)] for each SW is plotted versus dc ramp potential to obtain a peak-shaped voltammogram for an electroactive species. FFT–SWV is able to sample the current across the entire SW period and use a selected portion of the forward and reverse voltammogram to estimate the difference current considering that most of the current response was used to calculate the difference

current (i.e., 0–20% of the initial current rejected), the ΔQ was lower, possibly due to the charging component of the current interfering with the measurement of the faradic current.

Moreover, in this method different parts of voltammogram can be selected for a calculating the detector signal, based on response integration and it is one of the advantages of scanning approaches for electrochemical detection. A total absolute difference function (ΔQ) can be calculated by using the following equation.

$$\Delta Q(s\tau) = \Delta t \left[\sum_{E=E_i}^{E=Ef} \left| A(S,E)E - A(Sr,E)E \right| + \sum_{E=E_f}^{E=E_i} \left| A(S,E)E - A(Sr,E)E \right| \right] \quad (3)$$

where, s is the sweep number, τ is the time period between subsequent sweeps, Δt is the time difference between two subsequent points on the FFT–SW curves. E_i and E_f are the initial and the final potential, respectively, which are selected for integration of part of the voltammogram. The reference FFT–SW voltammogram was obtained by averaging a few voltammograms recorded at the beginning of the experiment (i.e., before injection of the analyte). The ΔQ adsorption-based response can be maximized by optimizing accumulation potential and time, the square wave amplitude and frequency and selecting suitable flow rate of the FFT–SWV response.

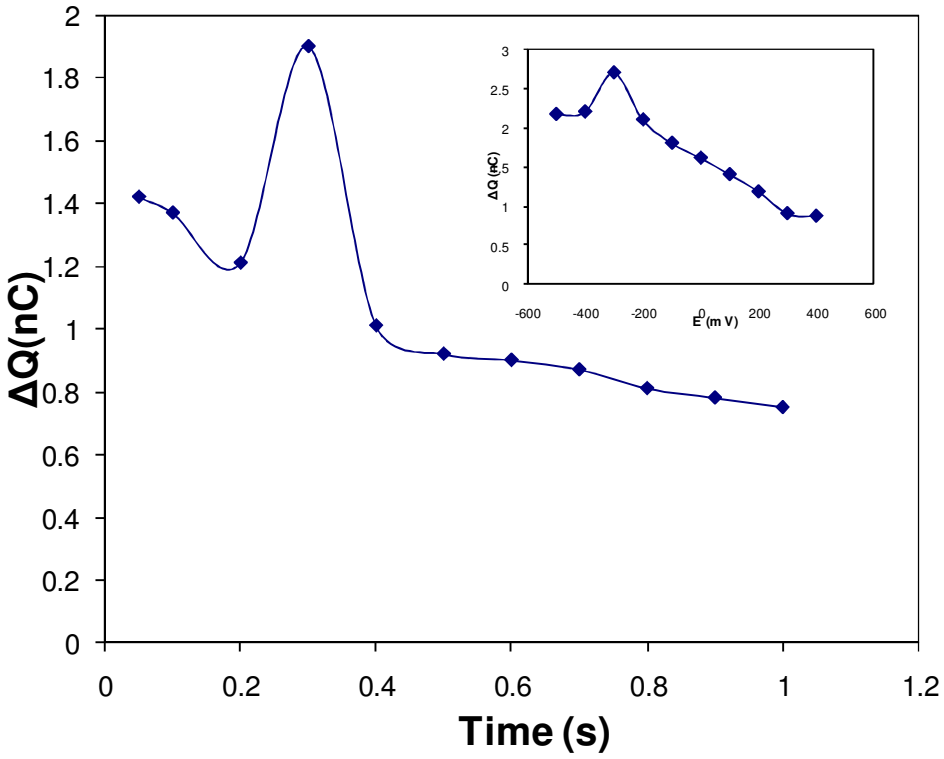


Figure 4. The effect of accumulation time and potential on the response of 25μm Pt UME to the injection of 5.0×10^{-6} mol/L Thiamine in 0.1 mol/L KHP.

3.1 Study of accumulation parameters

The dependence of the analyte signal on accumulation potential and time was studied at concentration level of Thiamine 5.0×10^{-6} mol/L. As expected, the extent pre-concentration is a function of the accumulation time (t_{acc}) and accumulation potential (E_s). As is shown in Fig. 4, the peak admittance increased with increasing accumulation time till 300 ms and after that it remains constant. For a period longer than 300 ms, saturation of the electrode surface by the analyte take places, and in shorter time the adsorbed analyte reduce sensitivity of the measurement. Hence, an accumulation time of 300 ms were chosen as the optimum value to evaluate the best sensitivity conditions to the proposed method.

The dependence of the peak current on the accumulation potential was evaluated over the range of -0.50 to 0.40 V for 5.0×10^{-6} mol/L of Thiamine at 0.1 mol/L KHP buffer pH 3.0 in the presence of the analyte, for an accumulation period of 300 ms. The results shown in the inset at Fig. 4, indicate that for an accumulation potential -0.30 V, the admittance values are maximum and after that the admittance decrease. As a result, after data analyzing the potential -0.30 V was chosen as a best accumulation potential.

3.2. Optimization of FFT-SW frequency and amplitude

The SW frequency and amplitude between 100–2000 Hz and 5–100 mV were examined respectively to study the effect of these parameters. In Fig. 5 the importance of frequency and amplitude is shown for solution of Thiamine. In fast voltammetric analysis, due to limitation in the rate of the electrode processes at the electrode surface, the SW frequency and amplitude are very important for sensitivity of the measurement. Since in this method, the analyte admittance and background noise, based on speed of applying the excitation potential.

In the measurement the solution resistance and electrode capacitance will limit the sensitivity enhancement that can be obtained by going to higher SW frequency. Nevertheless, increasing the SW frequency will increase the admittance peak of the adsorbed analyte, as well, the sensitivity of the measurements. But this will be moderated by a higher signal/noise ratio. Therefore, application of very high SW frequencies causes a shorter potential scan times, consequently, the response peak for the analysis becomes smaller and skewed, due to insufficient time for the processes occur at the electrode surface.

On the other hand, application of lower SW frequencies result to a longer potential scan times, which cause a lower number of potential scan for each injected sample zone, where produce inadequate data point in ΔQ -time peaks. For this reason, a series of SW frequencies were examined to determine the optimal frequency for the detection of Thiamine. A plot of SW frequency versus ΔQ showed that a frequency of 1200 Hz was the instrumental limit for this flow analysis. Thus, further studies of FFT-SWV detection used a frequency of 1200 Hz with a dc ramp time of 300ms to provide an overall sampling rate of 28 Hz.

To determine influence of SW amplitude on the analyte signal (ΔQ), various amplitudes were investigated. Fig. 5 shows the effect of SW amplitude on ΔQ . As is shown, ΔQ is increased with

increasing amplitude up to 60 mV, after which the value of ΔQ is plateau and then began to decrease when amplitudes greater than 60mV were used.

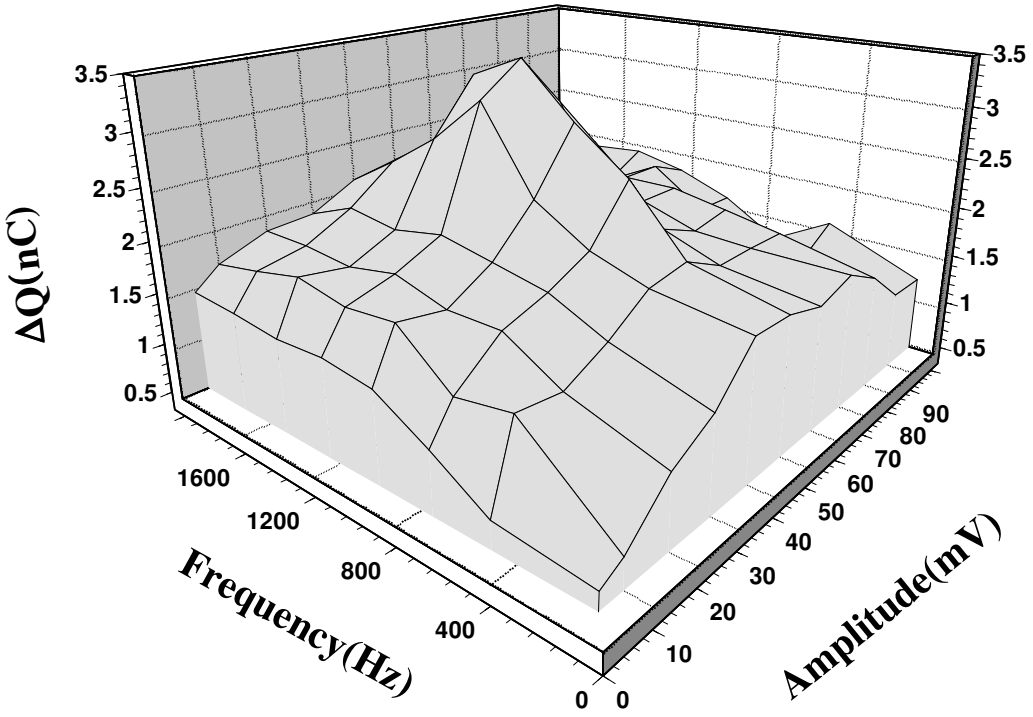


Figure 5. The effect of frequency and amplitude on the signal-to-noise ratio of Pt UME response to the injection of Thiamine in 0.1 mol/L potassium hydrogen phthalate.

3.3. Calibration curves

In all cases the experimental conditions were set at optimum values in order to obtain the best detection limits and the maximum S/N ratio which were the amplitude of 60mV and a frequency of 1200 Hz. As mentioned above the electrode response could be expressed in various ways as peak heights or peak areas in a selected range of the admittance volammogram. For this reason, the magnitude of injection peaks depends on the choice of the data processing methods The peak height (ΔQ *-time)* of Thiamine were plotted versus its concentration and linear regression analysis performed on the resultant curve. Due to this fact that there is a similarity between this method and striping voltammetry, consequently, the electrode response is proportional to the electrode coverage [25-31,34,35]. Therefore, the calibration curve constructed for Thiamine was linear over the concentration range of 5.0×10^{-10} to 4.0×10^{-7} mol/L. A correlation coefficient of R = 0.9989 with %R.S.D. values ranging from 0.30 to 2.5% across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be Y=4.6688x+0.3653 (R^2 = 0.9979). Calibration graph obtaining from the monitoring of Thiamine in a

0.1 mol/L KHP is shown in Fig. 6. The C_{DL} was measured as the lowest amount of the analyte that may be detected to produce a response, which is significantly (3 times) different from that obtained from the blank solution. Limit of detection was approved by calculations based on the standard deviation of the response (ı) and the slope (S) of the calibration curve at the levels approaching the limits according to equation C_{DL}= 3.3 (ı/S) [36]. The C_{DL} for Thiamine was obtained to be 8.0×10^{-11} mol/L. The LOQ was measured as the lowest amount of analyte that can be reproducibly quantified above the baseline noise. Consequently, the LOQ was 5.6×10^{-10} mol/L with a resultant %R.S.D. value of 1.4% (n = 5). To ensure the best S/N ratio, the measurements were carried out at high sweep rates.

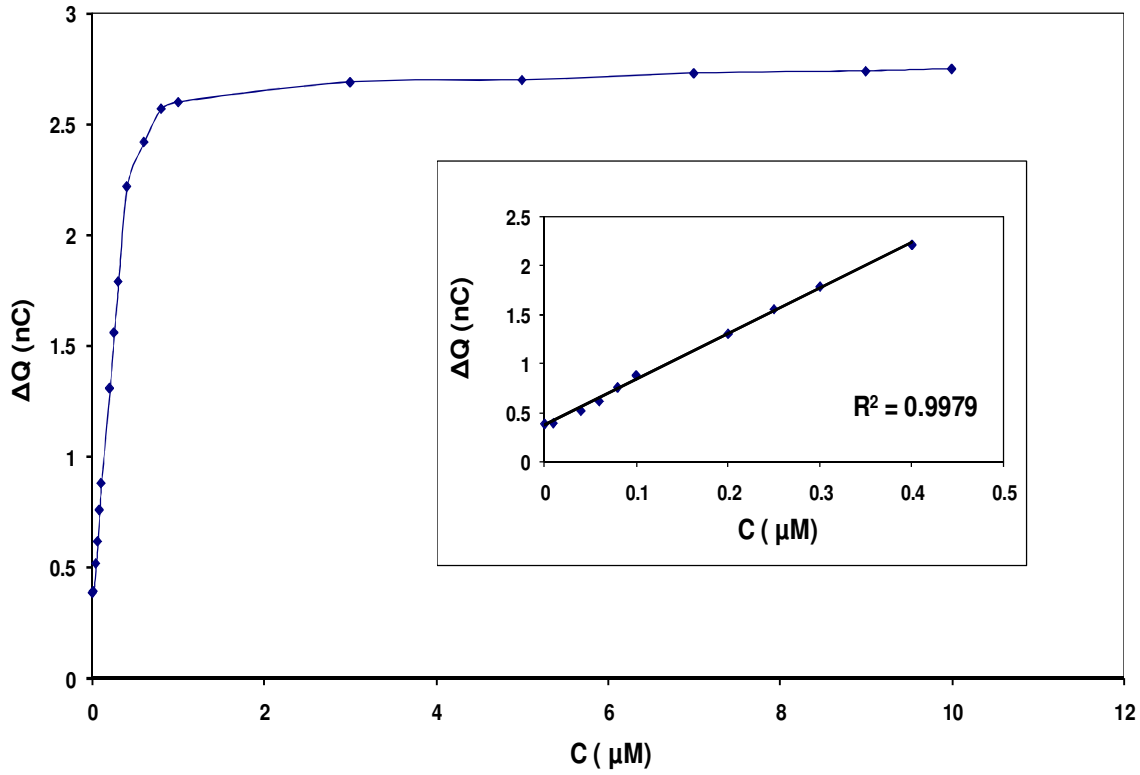


Figure 6. Calibration curve for Thiamine in 0.1 mol/L KHP under optimum condition at flow-injection system.

3.4. Ruggedness

The %R.S.D. values for intra- and inter-day assays of Thiamine in the cited formulations performed in the same laboratory by the two analysts did not exceed 4.0%, thus indicating the ruggedness of the method. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for Thiamine undertaken by two analysts. Also the robustness of the method

was investigated under a variety of conditions such as small changes in the pH of eluent, in the flow rate, in the buffer composition and in the laboratory temperature.

The result showed that the percent recoveries of Thiamine was between 98.9% and 101.8% which were good under most conditions and did not show a significant change when the critical parameters were modified.

3.5. Accuracy

Replicate (n = 6) peak areas of three accuracy standards (4.0×10^{-6}, 4.0×10^{-8} and 4.0×10^{-9} mol/L) from a calibration curve were interpolated as already mentioned. Also, the relevant error percentage and accuracy was calculated in each case. The resultant concentrations were $(3.7\pm0.3)\times10^{-6}$, $(4.1\pm0.3)\times10^{-8}$ and $(4.4\pm0.2)\times10^{-9}$ mol/L with percent relevant errors of 2.2%, 0.45% and 1.4%, respectively.

3.6. Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability of the measurement was investigated by injecting nine replicate samples of each of the 4.0×10^{-6}, $4.0\times10^{-8}$and 4.0×10^{-9} mol/L standards where the mean concentrations were found to be 4.2×10^{-6}, 4.4×10^{-8} and 3.6×10^{-9} mol/L with associated %R.S.D.'s of 1.8, 0.5 and 1.5, respectively. By injecting the same three concentrations over 3 consecutive days, inter-day precision was assessed resulting in mean concentrations of Thiamine of 4.1×10^{-6}, 3.8×10^{-8} and 4.5×10^{-9} mol/L and associated %R.S.D. of 2.1%, 0.8% and 1.9%, respectively.

3.7. Assay of tablets

The method worked in the present study was applied for the determination of Thiamine in tablets from the Iranian market resulting a percent recovery of 99.94% and a R.S.D. of 1.26%.

3.8. Sensitivity comparison of the detection methods

Finally, the sensitivity (detection limit) of this method is compared with those of the most sensitive previously reported methods in Table 1. As is obvious, the sensitivity of the method is superior to that of all previously reported methods. The data in Table 1 revealed that the detection limit of this method is at least about 10 times lower than that of other reported methods.

Table 1. Comparison between the detection limit of the proposed method and that of the other reported method

Detection limit	Reference
5.0×10^{-9} mol/L	[6]
2.0×10^{-9} mol/L	[7]
1.6×10^{-7} mol/L	[10]
6.6×10^{-10} mol/L	[11]
2.6×10^{-8} mol/L	[13]
8.0×10^{-11} mol/L	This work

4. CONCLUSIONS

This paper described a novel, sensitive, and widely applicable FFT–SWV FIA detection method using of UME platinum electrode. FFT–SWV was demonstrated to provide sensitive detection of a wide range of analytes based on oxidation of the electrode surface. The square-wave adsorptive voltammetry on a Pt ultramicroelectrode can be used to determine Thiamine at trace levels because of its high sensitivity. Currently, work is progressing on the enhancement of the detection electronics and FIA cell to allow incorporation of a second sensing electrode positioned away from the detection zone which will enable automatic, analog subtraction of the background response. It is hoped that this will make FFT–SWV easier to use as well as provide improved sensitivity. Also, application of FFT–SWV to high-performance liquid chromatography is being considered.

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