

Determination of Caffeine Content of Commercial Tea in Ethiopia

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Abstract: In this research, determination of caffeine content in ten commercial tea leaves available in Ethiopian markets have been reported using UV-Visible spectrometer and Square Wave Anodic Stripping Voltammeter. By applying both methods, the result show that, the highest content of caffeine was determined in Sirilanka black tea ($3.34 \pm 0.022\%$) where as Black Lion tea has the least caffeine ($1.88 \pm 0.015\%$). Even if the results obtained from both UV-Visible spectrometer and SWASV techniques give almost the same results, the results of SWASV technique using carbon paste electrode are in good agreement with the values obtained using HPLC method ($1.451 - 3.344\%$).

Keywords: Tea, Caffeine, UV-visible spectrometer, Square wave anodic stripping voltammetry and carbon paste electrode.

1. INTRODUCTION

1.1 Background of the study:

Tea refers to the agricultural products of the leaves, leaf buds and internodes of the tea (*Camellia sinensis*) plant. It has been consumed as a beverage for almost 2000 years starting in china [1]. It is the most widely consumed beverage after water [2].

There are two major types of tea, green tea and black tea [3]. Both contain caffeine 1 to 5 % of its dry weight depending on type, brand and brewing method. This is why the reported values in the literature are so variable [3], [4], [5].

Caffeine is an alkaloid; a class of naturally occurring compounds containing nitrogen and having the basic properties of organic amine. It belongs to a class of organic compounds called xanthine. Other common members of this class include theophylline and theobromine. It is found naturally in the leaves, seeds and fruit of tea, coffee, cacao, and more than 60 other plants worldwide [6].

Caffeine is used as a drug on the basis of its effect on respiratory, cardiovascular and the central nervous system. It is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. It is also included with ergotamine in some antimigrane preparations, the object being to produce a mildly agreeable sense of alertness [7].

A dose of 50–200 mg of caffeine is generally sufficient for a mild stimulation. Large amounts of caffeine can lead to intoxication, which results in flushing, chills, agitation, irritability, loss of appetite, weakness and tremor. Overdoses of caffeine may result in hypertension, hypotension, tachycardia, vomiting, fever, delusions, hallucinations, seizures, arrhythmia, cardiac arrest, coma and death [8], [9].

So, intake of large quantities of caffeine may cause a number of health problems. Tea is one of the most popular drinks in Ethiopia. It is suspected that due to the high frequency of intake of tea, the amount of caffeine consequently taken by people may be significantly high as to cause concern regarding the health problems mentioned. Therefore, knowing the content of caffeine in different tea brands is very important.

Determinations of the amount of caffeine in tea brands have been carried out in different parts of the world using different techniques. The latest and commonly used method of caffeine determination is based on the high performance liquid chromatography (HPLC). However, most reported procedures involve relatively long retention times or require lengthy sample pretreatments [10], [11]. There are also other techniques reported for caffeine determination such as Ion Chromatography, UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy, Electro-analytical Voltammetric and Nuclear Magnetic Resonance (NMR) Spectroscopy [12].

This research paper is intended to determine the amount of the caffeine content of commercial tea available in Ethiopia using UV-Visible spectroscopy and Square-Wave Anodic Stripping Voltammetric (SWASV) techniques.

1.2 Significance of the Study:

Tea is the most popular drink in Ethiopia. Due to the high frequency of intake of tea, the amount of caffeine consequently taken by people may be significantly increased. Different tea brands have different caffeine concentration based on variety of leaf, growing environment, application of fertilizers, manufacturing and infusion preparation. So, knowing the amount of caffeine content of commercial tea in Ethiopia gives hint for the user, which one to take on the basis of caffeine content. The aim of this study is to determine the levels of caffeine content of commercial tea brands available in Ethiopia.

2. MATERIALS AND METHODS

2.1 Apparatus and Instruments:

The instruments used for the experiments are electronic balance for measuring mass of different tea samples and caffeine, pH meter (model 3305), single beam UV-Visible spectrometer (model SP65, Gallenkamp, UK).

For electrochemical experiments SWASV was used in a three electrode electrochemical cell. The electrodes used are platinum coiled auxiliary electrode, Ag/AgCl reference electrode and carbon paste working electrode. The analyzer was BAS 100B connected to a Dell computer.

2.2 Chemicals:

The chemicals used for the experiment are: Caffeine (Fisher Scientific, analytical reagent), Chloroform (Winlab Limited, UK), paraffin oil (Abron Chemicals), graphite powder, phosphate buffer solution (Blulux laboratory reagent), NaOH (Blulux laboratory reagent), HCl (Blulux laboratory reagent), Na₂CO₃ (Blulux laboratory reagent) and anhydrous Na₂SO₄ (Blulux laboratory reagent). Samples and solutions were prepared using distilled water.

2.3 Samples:

In this investigation, ten different types of tea brand samples were used. Wush wush (black and green) tea, black lion black tea, good morning black tea, kokeb black tea, star black tea, ermon black tea and addis black tea samples are all Ethiopian products. Sirilanka green tea and black (mint) tea are both from Sirilanka. All tea samples are collected from different super markets.

2.4 Method of the Experiment;

i. Sample Preparation:

Sample preparation was performed by liquid-liquid extraction. Liquid-liquid extraction is a method to separate compounds based on their relative solubility's in two different immiscible liquids, usually water and an organic solvent like chloroform. It is an extraction of a substance from one liquid phase into another liquid phase. In this experiment, liquid-liquid extraction was applied based on the following procedures.

For UV-Visible spectrometer determination of caffeine: 30 mL of distilled water in a beaker was heated. 50 mg of tea leaves were immersed into the hot water by using spatula and stirred for 30 min with a magnetic stirrer. It was then cooled to room temperature and filtered through a filter paper. 40 mL of chloroform was poured into the tea infusion and stirred for 10 min. Since caffeine is more soluble in chloroform than in water, it readily dissolves in chloroform. The water phase and the chloroform phase were then separated by a separatory funnel. The volume of chloroform phase was measured. Some volume of the solution was taken into 1 cm cuvette and placed into the sample holder of the spectrometer and the absorption spectrum was then taken. These procedures were repeated three times for each different tea brands. The total content of caffeine in each of the tea samples was determined from the spectrum of combinations of the three extractions and calculated using Beer-Lambert laws from each individual absorption spectrum. The contents of caffeine in the fourth extraction were found negligible [2].

For electrochemical determination of caffeine: 5 g of tea sample, 100 mL distilled water and 2 g of Na_2CO_3 were added into a 250 mL Erlenmeyer flask. The mixture was boiled in a hot plate with stirring till Na_2CO_3 dissolved. The mixture was allowed to cool for 5 minutes, and then decanted into another beaker and continue cooling up to room temperature of laboratory class (20 – 23 °C). 100 mL of chloroform was added to the solution and mixed very well. Using separatory funnel, caffeine was extracted from solution. The extraction was passed through 0.25 g of anhydrous Na_2SO_4 for removing water of the tea. Chloroform was left to evaporate and the residue was dissolved in 10 mL of supporting electrolyte (0.1 M phosphate buffer) [13].

ii. Preparation of Working Electrode:

Carbon paste electrode (1 g) was prepared by mixing graphite powder with paraffin oil. The composition of the paste was 70% (w/w) graphite powder and 30% (w/w) paraffin oil. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hrs. The homogenized paste was packed in to the tip of a plastic tube (chewing gum stick, which is bought from ordinary shop and it has a diameter of 3.5 mm). A copper wire was inserted from the backside of the syringe to provide electrical contact. The surface of the electrode was smoothed manually against a smooth white paper until a shiny surface is emerged [14].

3. RESULTS AND DISCUSSION

3.1 Determination of Caffeine Content using UV-Visible Spectrometer:

i. Calibration Curve:

To determine the molar absorption coefficient of caffeine in chloroform, different concentrations of pure caffeine were prepared and absorption spectra were recorded using UV-Visible spectrometer. The UV-Visible absorbance range of caffeine is from 245.5 to 305 nm in chloroform. The peak absorbance of each concentration of the absorption spectra of pure caffeine in chloroform solution is at peak wavelength of 276 nm.

The absorbance versus wavelength graph of the absorption curve for different concentration of pure caffeine is shown in Fig. 1.

From the measured value of absorbance and concentration of pure caffeine, the values of molar absorption coefficient (ϵ) were determined. The summarized value is shown in table 1.

TABLE 1. Physical quantity of absorption spectra of different concentrations of pure caffeine with its molar absorption coefficient in chloroform

Absorbance	Concentration(mM)	$\epsilon(\text{cm}^{-1}\text{M}^{-1})$
0.139	0.021	66.2
0.170	0.025	66.3
0.177	0.027	65.6
0.341	0.052	65.6
0.452	0.069	65.5
0.817	0.122	67.0

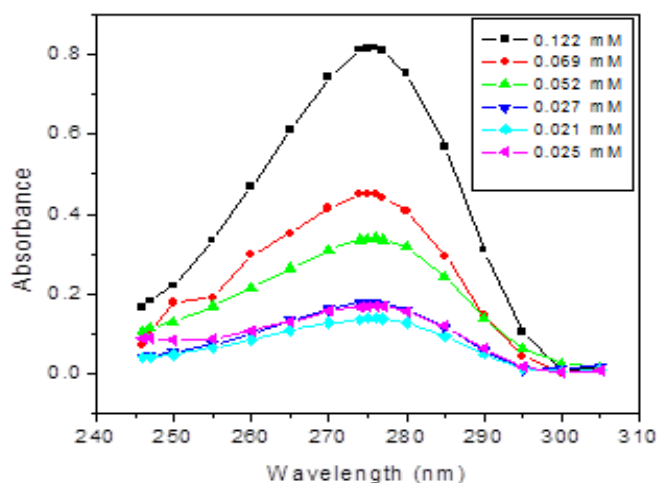


Fig.1. Absorbance vs wavelength of different concentrations of pure caffeine using chloroform as solvent

The value of molar absorption coefficient of pure caffeine in chloroform is $66.0 \text{ cm}^{-1}\text{M}^{-1}$. In electronic spectroscopy especially in organic molecules the transition observed in UV-Visible region is $\pi^* \leftarrow \pi$. Then for pure caffeine the electronic type transition is $\pi^* \leftarrow \pi$ transition and this transition is the cause for the observed absorbance.

The concentration versus absorbance graph for the quantities described in table 1 is shown in Fig. 2.

ii. Validation of the method:

As shown in Fig. 2, the calibration graph correlating to the absorbance and concentration of pure caffeine in chloroform was constructed at the λ_{max} for concentration range of 0.025 – 0.122 mM. The standard deviation is 0.00522 and the linear regression coefficient is 0.99984. From Fig. 2, good linear relationships were observed for a wide concentration range in agreement with Beer Lambert law.

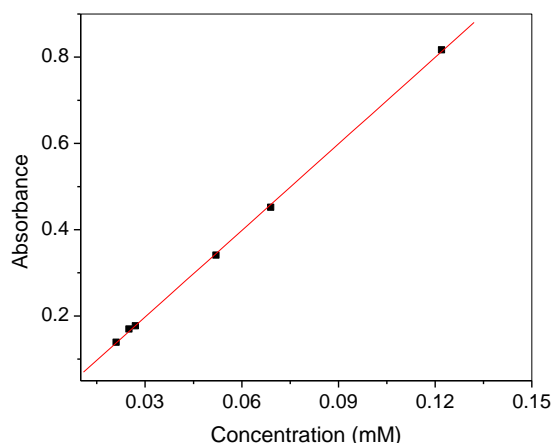


Fig. 2. Absorbance vs Concentration graph for pure caffeine in chloroform

iii. Determination of Caffeine in Tea Samples:

Due to the matrix effect of UV absorbing substances, spectrometer method cannot determine caffeine directly from the sample using water as solvent [15]. This effect has been observed in the spectral bands of tea leaves in water as shown in Fig. 3. A caffeine spectrum was interfaced with other. Therefore it is necessary to first dissolve caffeine in water and then extract it using chloroform as mentioned in the procedure part [2].

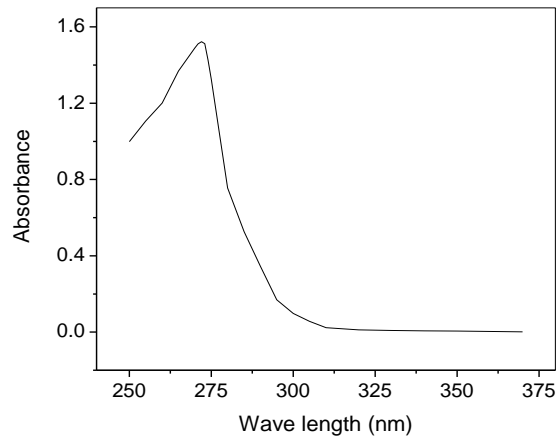


Fig. 3. Absorbance vs wavelength of caffeine extracted from tea leaves using water as solvent

In Fig. 4, the absorption spectra of 0.11 mM pure caffeine and caffeine extracted from wash wash black tea by chloroform exactly overlaps. This indicates that chloroform was a good solvent used to extract caffeine from different tea brands.

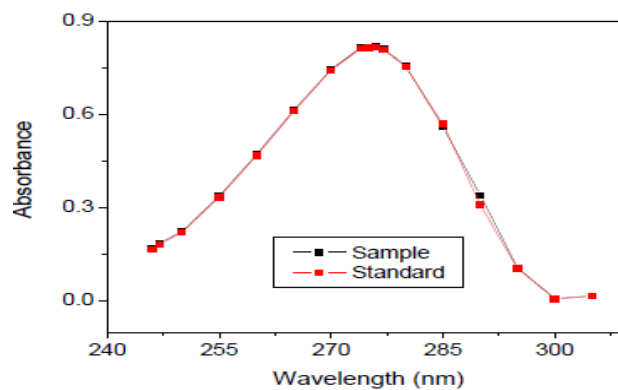


Fig. 4. Overlay of UV-Visible spectra of 0.11 mM pure caffeine and caffeine extracted from wash wash black tea in chloroform

To determine caffeine content from different tea brands, the extractions were repeated four times until the absorbance of caffeine becomes flat. In the fourth extraction, as observed in Fig. 5, the content of caffeine was found negligible.

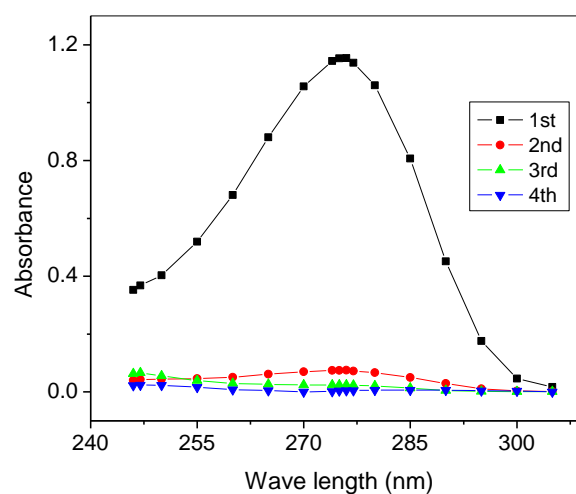


Fig. 5. Absorbance vs wavelength of caffeine for different round of extraction for the 1st, 2nd, 3rd and 4th extraction in chloroform

Using absorbance and molar absorption coefficient, the caffeine concentration of the sample was determined at each extraction. From measured volume of the solution, mass of caffeine was calculated and changed into percent by mass (w/w). The result of this indicates that most caffeine was extracted during the first extraction (about 90 – 95%, 3.5 – 9%, 0.5 – 2% and 0% for 1st, 2nd, 3rd and 4th round extractions respectively) of the total caffeine. The quantity of caffeine in the tea brands was determined based on this method and was found between 2.06 - 3.14% (w/w) and presented in table 2.

TABLE 2. Experimental results of caffeine content of different tea brands using UV-Visible Spectrometer

Sample	Percent of caffeine (w/w) \pm SD
Sirilanka Black Tea (Mint)	3.10 \pm 0.023
Wush Wush Black Tea	2.93 \pm 0.026
Good Morning Tea	2.77 \pm 0.044
Kokeb Black Tea	2.76 \pm 0.026
Ermon Black Tea	2.71 \pm 0.014
Wush Wush Green Tea	2.56 \pm 0.041
Addis Black Tea	2.30 \pm 0.041
Star Black Tea	2.00 \pm 0.014
Sirilanka Green Tea	1.08 \pm 0.021
Black Lion Tea	1.06 0.031

3.2 Determination of Caffeine Content using SWASV:

The electrochemical behavior of caffeine on carbon paste electrode was studied by square wave anodic stripping voltammetry (SWASV) using a potential range from 0.0 - 1.7 V with 0.1 M phosphate buffer.

The effect of square wave voltammetric parameters was examined on the peak current height of caffeine. The basic parameters are determined as follows:

i. Effect of pH on Peak Current:

The influence of pH on the oxidation peak current and peak potential of caffeine was investigated in the pH range of 6 - 12 using 1.0 mM caffeine in 0.1 M phosphate buffer. The effect of pH on the peak current is shown in Fig. 6. The peak current was slightly increased from pH 6 to pH 7.5, reaching a maximum at pH of 9.1 and decreasing in higher pH ranges. The highest peak current, pH = 9.1, was chosen for the present work. The influence of pH on the peak potentials of caffeine was also examined. The peak potentials showed a shift to the negative direction with the increase in pH of the supporting electrolyte.

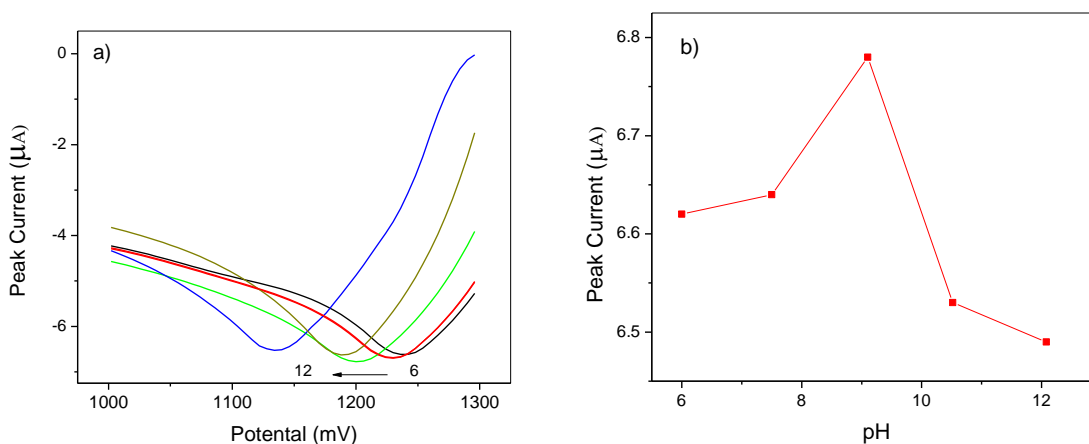


Fig. 6. a) SW stripping voltammograms of caffeine at different pH (6, 7.5, 9.1, 10.5 & 12.08); b) SW voltammetric peak current at various pH for 1.0 mM of caffeine; with initial potential of 0 V, final potential of 1.70 V, quiet time of 2 s, amplitude of 40 mV, frequency of 25 Hz, and step potential of 6 mV

ii. Effect of Deposition Time on Peak Current:

Since the stripping voltammetric currents are dependent on the various stripping and SW parameters, this study investigated the best parameters that would maximize a stripping current when caffeine is deposited at 0.0 V potential then stripped at a more positive potential [14]. In Fig. 7, the effect of deposition time was studied between 0 - 180 s at 30 s intervals. As expected, the peak current increases as the pre-concentration time increases. Specifically, it increases at a fast rate up to about 120 s, increasing rather slowly at a range between 120 - 180 s. Thus, 120 s was taken to be the optimum accumulation time for pre-concentration prior to stripping.

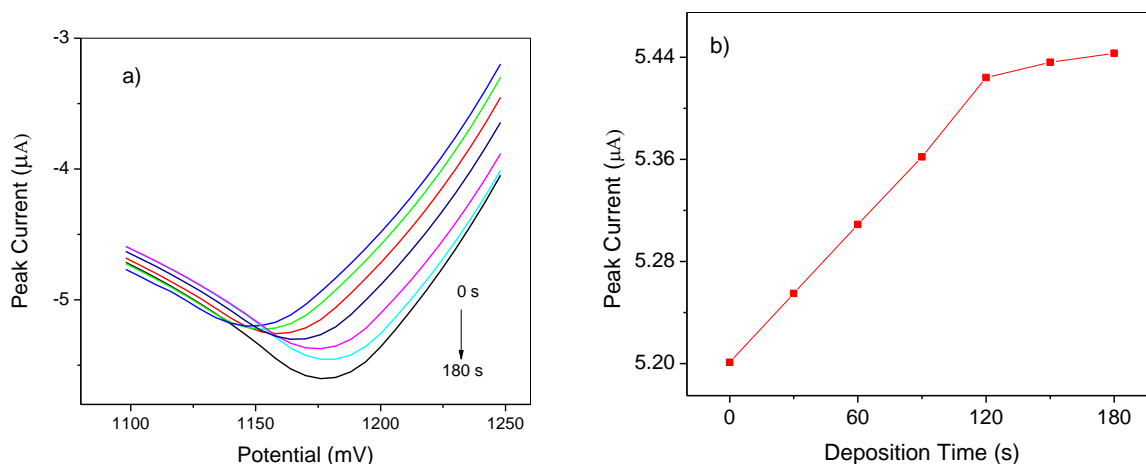


Fig. 7 a) SW stripping voltammograms of caffeine at different deposition times (0, 30, 60, 90, 120, 150 & 180 s); b) SW voltammetric peak current at various deposition times for mM caffeine; where other experimental parameters in Fig. 6 were held constant

iii. Effect of Frequency on Peak Current:

As can be seen from Figure 8, the magnitude of the peak current increases with increasing SW frequency, however the slope changes to a higher value after frequency of 25 Hz. This indicates that the dependency of the peak current on frequency is being distorted due to an accompanying peak broadening. In addition, in the experiment conducted the peak current was unstable at high frequencies and which affects the reproducibility of the measurement. As a result, 25 Hz was chosen as the SW frequency for the subsequent experiments.

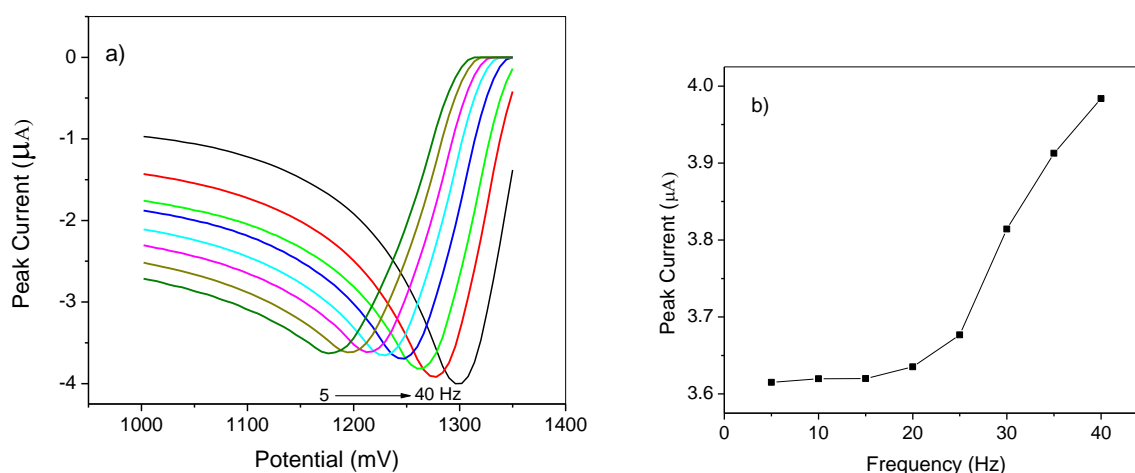


Fig. 8a) SW stripping voltammograms of caffeine at different Frequencies (5, 10, 15, 20, 25, 30, 35 & 40 Hz); b) SW voltammetric peak current at various frequencies for 1.0 mM caffeine; where other experimental parameters in Fig. 6 were held constant

iv. Effect of Amplitude on Peak Current:

Fig. 9 illustrates the stripping peak current of caffeine as a function of the SW amplitude at 1.0 mM caffeine concentration, after a pre-concentration time of 120 s. The SW amplitude was studied between 5 - 55 mV at 5 mV intervals. As expected, the peak current increases linearly as the amplitude increases. At amplitudes higher than 40 mV, however, peak broadening becomes much larger. Thus, 40 mV was chosen as the optimum pulse height to compromise the positive effect of the peak height and the negative effect of widening of the peak width.

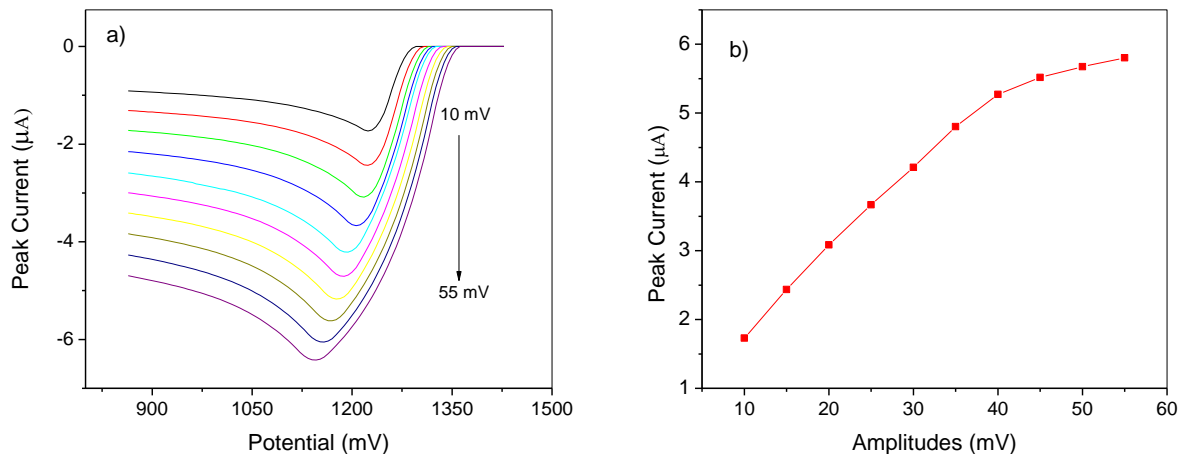


Fig. 9 a) SW stripping voltammograms of caffeine at different Amplitudes (10, 15, 20, 25, 30, 35, 40, 45, 50 & 55 mV); **b)** SW voltammetric peak current at various amplitudes for mM caffeine; where other experimental parameters in Fig. 6 were held constant

v. Effect of Step potential on Peak Current:

The last parameter investigated for square wave voltammetric measurements was the step potential. The effect of step potential (2–11 mV) on the peak height of 1.0 mM caffeine is depicted using 40 mV SW amplitude and the other experimental parameters as in Fig. 10. The peak current increases as the SW step potential increases, however, accompanied by peak broadening and affecting the reproducibility of the detection. So, for further work a step potential of 6 mV was selected.

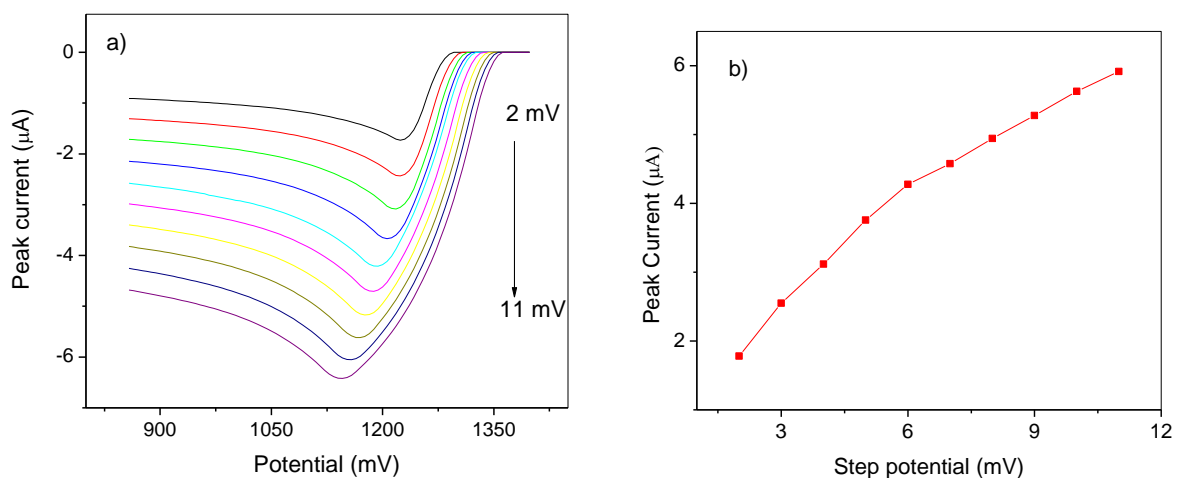


Fig. 10 a) SW stripping voltammograms of caffeine at different step potentials (2, 3, 4, 5, 6, 7, 8, 9, 10 & 11 mV); **b)** SW voltammetric peak current at various Sw step potential for mM caffeine; where other experimental parameters in Fig. 6 were held constant

The optimum parameters used for the experiment are summarized in Table 3.

TABLE 3. Optimum values of the experimental parameters for SWASV technique used to determine the caffeine content of different tea brands in this study

Parameters	Optimum values
pH of the supporting electrolyte	9.1
Square wave deposition time (s)	120
Square wave frequency (Hz)	25
Square wave amplitude (mV)	40
Square wave step potential (mV)	6

vi. Effect of concentration on Peak Current and Linear Range

Fig. 11a displays raw voltammograms at various caffeine concentrations with the optimum conditions. After the background current was subtracted from the measured currents, the adjusted currents were plotted like Fig. 11b. This exhibits the linear range in the concentration range of 2.0 – 10.0 μM . A linear regression yield an equation of $i_p = 4.3667 + 208c$ (i_p is peak current in μA and c is concentration in μM) with a correlation coefficient of 0.99729 and standard deviation (SD) of 0.0005051. The linearity can be used for the determination of caffeine concentrations in the samples.

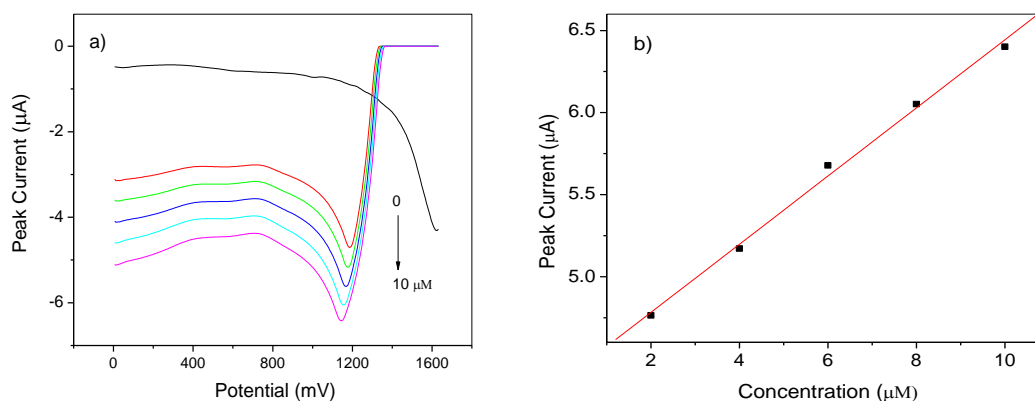


Fig. 11 a) SW stripping voltammograms of caffeine at different concentrations (0.0, 2.0, 4.0, 6.0, 8.0, & 10.0 μM); b) The calibration curve with the results of regression and SD; at optimum conditions, with initial potential of 0.0 V and final potential of 1.7 V

vii. Determination of Caffeine in Tea Samples:

The quantity of caffeine in the different samples was determined using the calibration curve of Fig. 11b. The results are presented on table 4.

The result indicates that the percentage of caffeine (w/w) in sirilanka black tea (mint) is the greatest (3.34 ± 0.000502) of all the other tea brands studied while black lion tea has the least caffeine content (2.08 ± 0.00051).

TABLE 4. Experimental results of caffeine content of different tea brands by using SWASV

Sample	Percent of caffeine (w/w) \pm SD
Sirilanka Black Tea (Mint)	3.34 ± 0.022
Wush Wush Black Tea	3.02 ± 0.011
Good Morning Tea	2.79 ± 0.034
Kokeb Black Tea	2.79 ± 0.012
Ermon Black Tea	2.74 ± 0.005
Wush Wush Green Tea	2.56 ± 0.012
Addis Black Tea	2.37 ± 0.023
Star Black Tea	2.06 ± 0.031
Sirilanka Green Tea	1.91 ± 0.024
Black Lion Tea	1.88 ± 0.015

Caffeine content in tea leaves reported in this research is in the range of values reported by other researchers (1 to 5%).²⁻⁴

Even if the results obtained from both UV-Visible spectrometer and SWASV techniques give almost the same results, the results of SWASV technique using carbon paste electrode are in good agreement with the values obtained using HPLC method (1.451 – 3.344 %) [2], [15], [16].

4. CONCLUSION

Caffeine content of different brands of tea has been determined using UV-Visible spectrometer and SWASV techniques. The highest content of caffeine was found in sirilanka black tea (mint) (3.34%), where as black lion tea was characterized with the lowest content of caffeine (1.88%).

SWASV using carbon paste electrode can be an alternative to the HPLC method (which is a commonly used method for the analysis of caffeine content) [16].

Finally, further studies must perform by the researchers, in order to determine the amount of caffeine used by the user in each cup of tea.

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