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Quantification of Sub-Nanomolar Levels of Gallic Acid by Adsorptive Stripping Voltammetry

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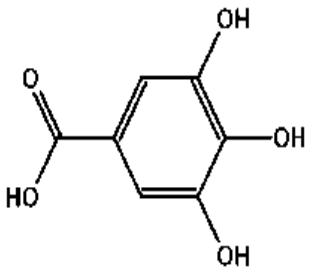
The voltammetric behavior of gallic acid (GA) on a hanging mercury drop electrode (HMDE) has been investigated by cathodic adsorptive stripping voltammetry (AdCSV). Gallic acid (3,4,5-trihydroxybenzoic acid) is a natural phenolic compound which can generate a well defined reduction peak at around -1.04 V (versus Ag/AgCl) in 0.1 M borax buffer solution (pH 7.53). The parameters affecting the response of gallic acid, such as pH, accumulation time, accumulation potential and scan rate are optimized for the determination of GA. Under the optimized conditions, the peak current change linearly with the GA concentration in the range of 0.1 to 600 ng/ml. The lowest detectable concentration of GA is 0.05 ng/ml after 100 s accumulation time. The method has been successfully applied to the determination of GA in tea, urine and plasma blood serum. The results show that the sensitivity of the method is highest among the existing methods.

Keywords: Gallic acid; Adsorptive stripping voltammetry; Antioxidant

1. INTRODUCTION

Gallic acid (GA; 3, 4, 5-trihydroxybenzoic acid), is a natural polyphenolic compound whose structure is shown in scheme 1. Phenolic acids are aromatic secondary plant metabolites, widely spread through the plant kingdom [1]. The basic feature of phenolic acids is the presence of one or more hydroxylated benzene rings. High gallic acid content can be found in gallnuts, grapes, sumac, witch hazel, tea leaves, hops, and oak bark. Gallic acid exists in two forms as the free molecule and as part of tannins. Pure gallic acid is a colorless crystalline organic powder, while salts and esters of gallic acid are termed gallates. It has many applications in chemical research and industry such as being used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay and also used for making dyes and inks. Gallic acid is commonly used in the pharmaceutical industry because

many in vivo and in vitro studies in human, animals, and cell culture have provided evidence for the following actions of gallic acid. It shows cytotoxicity against cancer cells, it can be used to treat albuminuria and diabetes [3], it seems to have antifungal and antiviral properties [4], used as an antioxidant and helps to protect human cells against oxidative damages [5]. It can be used as a remote astringent in cases of internal hemorrhage [6], used to treat psoriasis and external hemorrhoids containing gallic acid. It can be used as clarifying agent in the brewing and wine industry, and as flavoring agent in foods, without harming healthy cells black foods, candy and meat products [2]. Bound forms of GA, notably epicatechin gallat or galloyl glucosides and tannic acids are associated with and are probably the main source of free GA in food and natural products [2]. Pharmacological investigations performed so far on GA have shown that this substance have several biological activities such as antimicrobial [3], anti-inflammatory [4], antioxidant [5-6], anticarcinogenic [7-10], and ant mutagenic [6, 8] activities. A number of methods have been developed for the determination of GA and other phenolic compounds in biological materials. The determination of GA is of interest in food, medicine, and other industrial products. GA is also toxic to animals probably because of its reaction with macromolecules such as DNA or protoglycans. Various methods for determination of GA and other phenolic compounds in biological materials have been reported using paper chromatography [15-18] and thin – layer chromatography [18-20], but these methods are nonspecific and nonselective. In this work we have developed a simple, rapid and inexpensive method for the determination of gallic acid.



Scheme 1. Chemical structure of gallic acid

2. EXPERIMENTAL

2.1. Chemical and reagents

All chemical reagents were of analytical grade, purchased from Merck (Germany). All solutions were prepared with de-ionized water. A stock standard solution of gallic acid (1×10^{-3} M) was prepared by dissolving 0.0189 g of gallic acid in 100 ml volumetric flask. More dilute solutions were prepared by diluting this solution with water. Borax buffer solution was prepared using boric acid and sodium hydroxide.

2.2. Apparatus

All polarographic measurements were carried out using a polarographic processor, model 746 VA (Metrohm), in combination with a polarographic stand model, 747 VA (Metrohm).The electrode

stand consist of a hanging mercury drop electrode (HMDE) as working electrode, a double junction Ag/AgCl (3M KCL, saturated AgCl, and 3M KCL in the bridge) as reference electrode and platinum wire, with considerably larger surface area than that of HMDE, as auxiliary electrode. All potentials quoted are relative to Ag/AgCl reference electrode. Stirring was carried out by a large Teflon road with 2000 rpm speed. A 780 pH Meter (Metrohm), equipped with a combined Ag/AgCl glass electrode was used for pH measurements. Eppendorf reference variable micropipettes were used to pipette microlitre volume of solutions.

2.3. Sample preparation

2.3.1. Determination of gallic acid in plasma and urine

Exactly 2.0 ml of whole blood sample were transferred to each test tube and left to equilibrate in a water bath for 1 h at 37 ºC. After that, 1.0 ml water was used to quantitatively transfer the sample into Teflon high- pressure microwave acid digestion vessels for the digestion. 2.0 ml of concentrated nitric acid and 4 ml of 30% H_2O_2 were added. After digestion of blood samples, the closed vessels were cooled; the digests were quantitatively transferred to a 10.0 ml volumetric flask, neutralized with 1.0 M NH_3 and diluted to volume with distilled water [11]. Volumes of 1.0 ml of each of aliquots were taken for the determination of gallic acid via the recommended procedure.

2.3.2. Determination of gallic acid in tea samples

200 mg of the sample was weighted into a test tube. The sample was extracted with 5 ml methanol or ethanol in ultrasonic bath for 45 minutes. Then the samples were centrifuged for 7 minutes at 4200 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25, transferred into vial prior analyses.

2.4 Measurement procedure

The mixture solution (contain gallic acid and buffer solution) was stirred by purging for 3 min with water – saturated nitrogen gas prior to analysis. The nitrogen flow was stopped and a new mercury drop was extruded, and adsorption potential was set to + 50 mV for 100 s, while the solution was stirred 2000 rmp. Then the stirred was switched off and a quiescence time of 10 s was allowed. The potential scan was carried out from +100 to -120 mV and voltammograms were recorded using differential pulse modulation with a potential scan rate of 60 mV/s.

3. RESULTS AND DISCUSSION

Voltammetric response of GA on HMDE

The typical voltammogram of GA is shown in Fig.1. GA can produce a reduction peak at -1.04 V on HMDE electrode. The electron- donating ability of gallates, which are food and pharmaceutical

antioxidants, is quantitatively assessed on the basis of their electrochemical characteristics [14]. Gallic acid has pK_a values of .4, 8.2, 10.7 and 13.1 [15].Gallic acid was electrochemically oxidized on the hanging mercury drop electrode by using differential pulse voltammetry. This compound is easily oxidized in neutral solution. Electrochemical oxidation occurs via two electron transfer steps, the oxidation process found to be irreversible, diffusion controlled and pH-dependant. The peak has a high sensitivity. The oxidation peak potential of the phenolic hydroxyl group of GA varied linearly with increasing pH value of solution. It could be interfered that the oxidation procedure of GA related to H^+ ions of solution and the same number of protons and electrons participated in the oxidation process of GA.

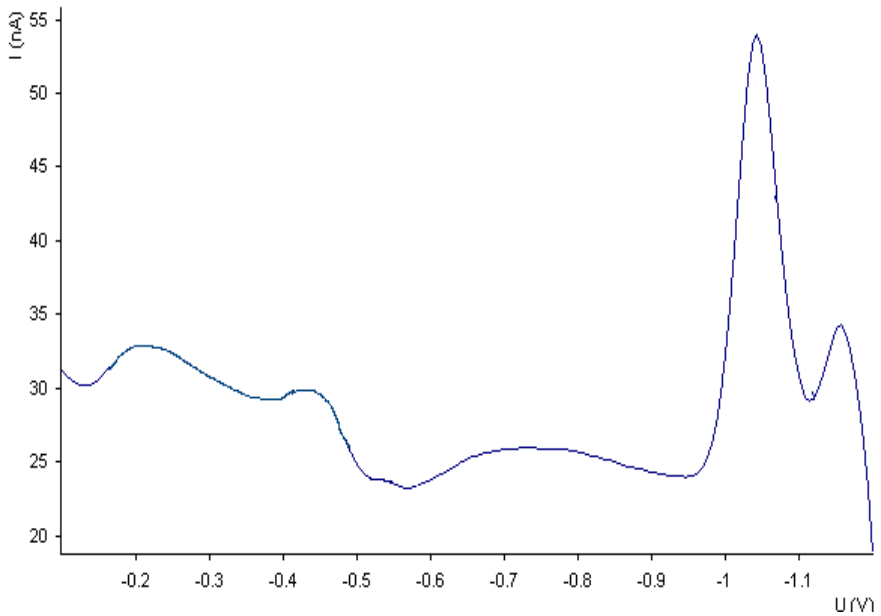


Figure 1. Differential plus voltammogram for determination of GA. Conditions: 1×10^{-7} M GA, initial potential, +100 mV: final potential, -1200 mV; accumulation potential, +50 mV; accumulation time, 50 s and sweep rate, 60 mV/s.

This result was similar to previous work reported by Gunckel et al. [14]. Furthermore, it was observed that the oxidation peak current of GA (I_{pa}) varies linearly with ν (scan rate) rather than $\nu^{1/2}$. The result indicted that the electrode process was controlled by adsorption step [16]. The structure of GA has 3 functional OH groups, which can undergo oxidation or reduction and its activity does not reside only in these groups and is enhanced by the carbonyl group. The reduction of GA studied by stripping voltammetry at pH 7.5 showed a reduction peak at -1.04 V .The peak current was smaller in acid and alkaline media and increased at neutral pH. As shown in Fig.2, the plot of differential pulse voltammograms as a function of pH. As can be observed, the compound exhibit a very good resolved voltammetric peaks at -1.04 V. From the shape of the main peak, we can see a sharp decrease in the base line before and after the peak. This phenomenon can be ascribed to a strong change in the charging current probably due to adsorption effects.

3.1. Effect of buffer solution and influence of pH

Different buffer solutions were tested for their suitability in determination of GA, as follows: $NaHCO_3$ – NaOH, borax – NaOH, K_2HPO_4 – NaOH, NH_3 – NH_4Cl, CH_3COONa – CH_3COOH. The most suitable buffer system for the determination of GA was found to be borax – NaOH buffer. In this buffer solution, peak shape and height is well defined. The influence of pH on the voltammetric response of gallic acid was examined in a borax buffer aqueous medium. The effect of variation of pH on the differential pulse voltammogram of gallic acid is shown in fig 2. The results obtained clearly showed that protons are involved in the oxidation process of gallic acid on HMDE. The variation of peak current with pH is shown in fig.2. Within the range of 5.8 to 7.5 increases linearly until attaining a maximum at about pH 7.53 and then decreases. When pH is above 8.5 the reduction peak almost disappears. It is related to proton taking part in the electrochemical reduction. In basic solutions the reactions become difficult because of the short of proton. The peak potential shifts negatively with increasing pH.

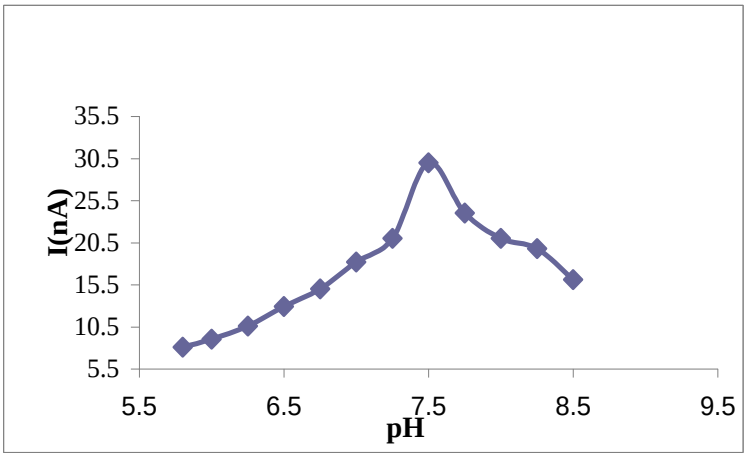


Figure.**2**. Dependence of peak heights on the pH value. Conditions: 1×10^{-7} M GA, accumulation potential +50 mV; accumulation time 50 s and sweep rate 60 mV/s.

3.2. Influence of accumulation potential and accumulation time

As shown in Fig.3, by increase of accumulation time, the peak current increased rapidly until become relatively stable at around 100 s, after which it change slightly. This reflects the deposition–controlled feature of the electrochemical process. When the accumulation potential changed from +250 to +100 mV, the peak current kept almost unchanged, indicating that the accumulation is almost independent of electrostatic attraction. This is in agreement with the neutral nature of GA in such pH circumstances.

As GA and HMDE are hydrophilic (i.e. –COOH, -OH) the accumulation of GA on HMDE should be ascribed to the molecular interaction between them, such as hydrogen bond. When the GA concentration is increased furthermore, the increase rate of peak area lowers and at last the peak area

kept almost unchanged. This indicated that the adsorption of GA onto HMDE basically belong to Langmuir adsorption.

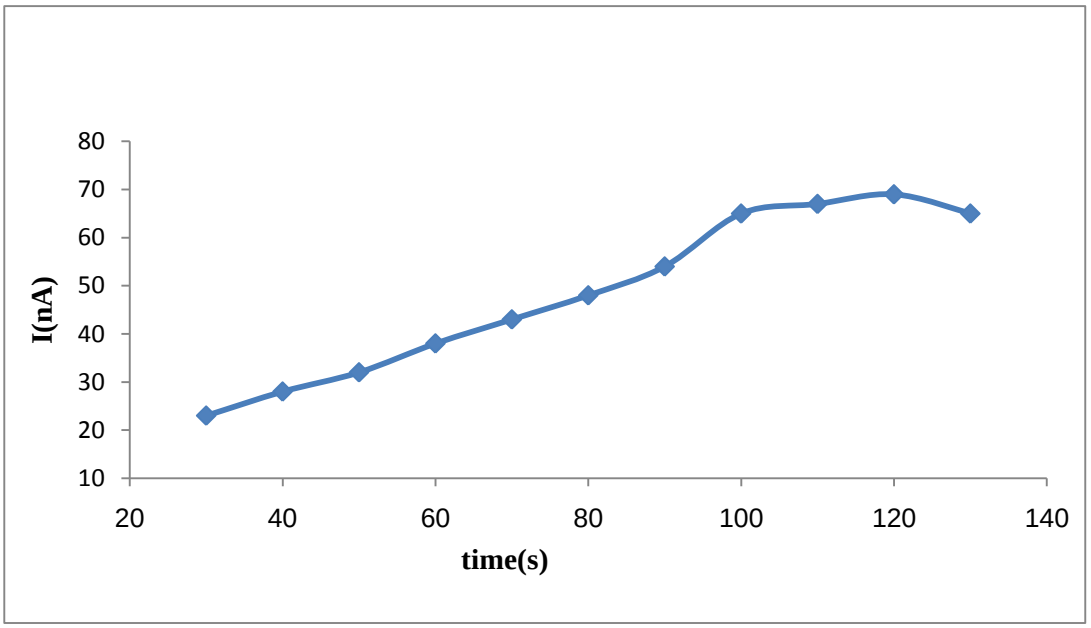


Figure 3.Peak heights in dependence on the accumulation time. Conditions: : 1× 10^{-7} M GA, accumulation potential, +50 mV; sweep rate, 60 mV/s.

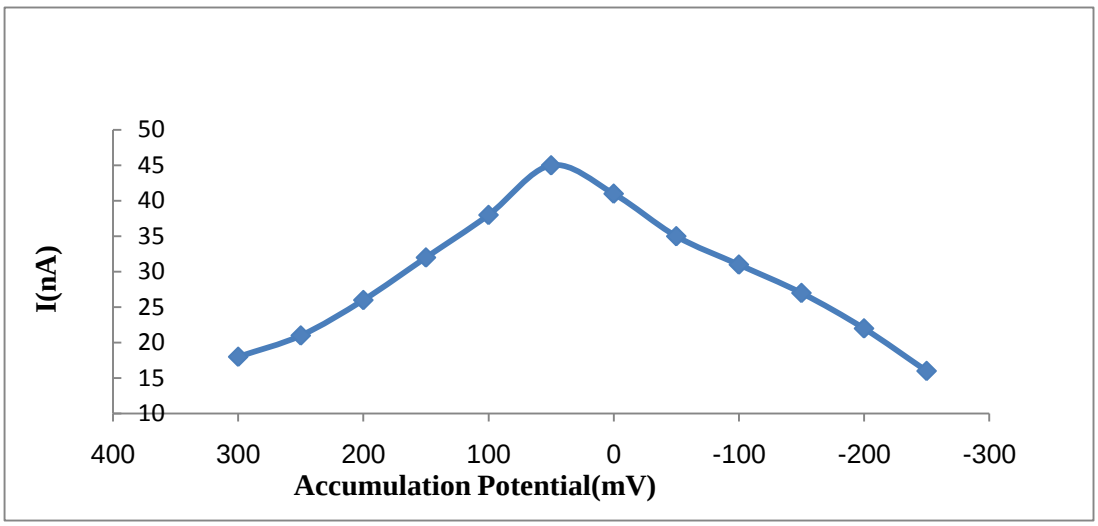


Figure 4. Peak heights in dependence on the accumulation potential. Conditions: : 1× 10^{-7} M GA, accumulation time, 50 s and sweep rate, 60 mV/s.

3.3. Influence of scan rate

Peak current and scan rate show a good linear relationship over the range of 20mv/s to 80 mV/s (Fig.5). This indicated that electrochemical process of GA is controlled by adsorption.

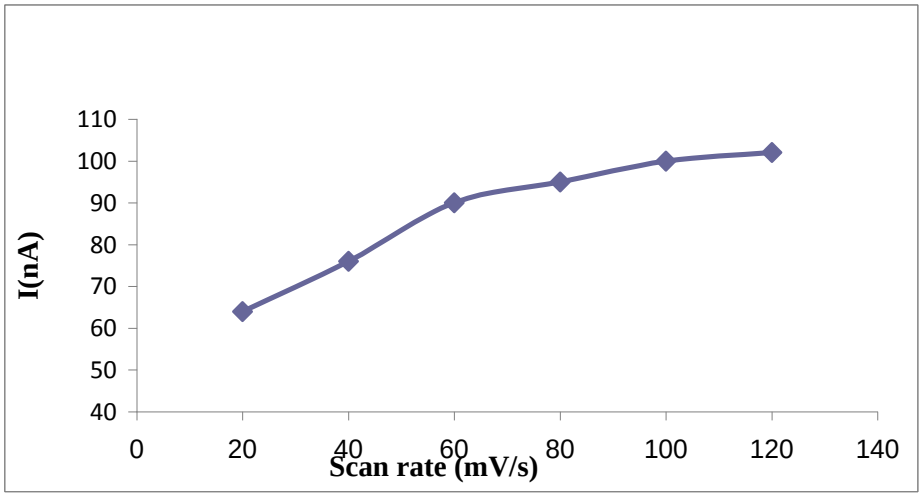


Figure. 5. Effect of Scan rate on the peak current. Conditions: 1×10^{-7} M GA, accumulation potential, +50 mV; accumulation time, 100 s.

3.4. Calibration curve and stability

Under optimized conditions, the reduction peak is linear to GA concentration over the range of 0.1 to 600 ng/ml (see fig. 6). The regression equation is I_p= 1.088C+5.488, (I_p(μA), C(ng/ml), r^2=0.999).When the concentration is increased further the I_p versus C deviated from the plot, resulting from decrease of accumulation efficiency. The lowest detectable concentration of GA is estimated to be 26.5×10^{-11} M after 100 s accumulation time. This indicated that the method is quite sensitive.

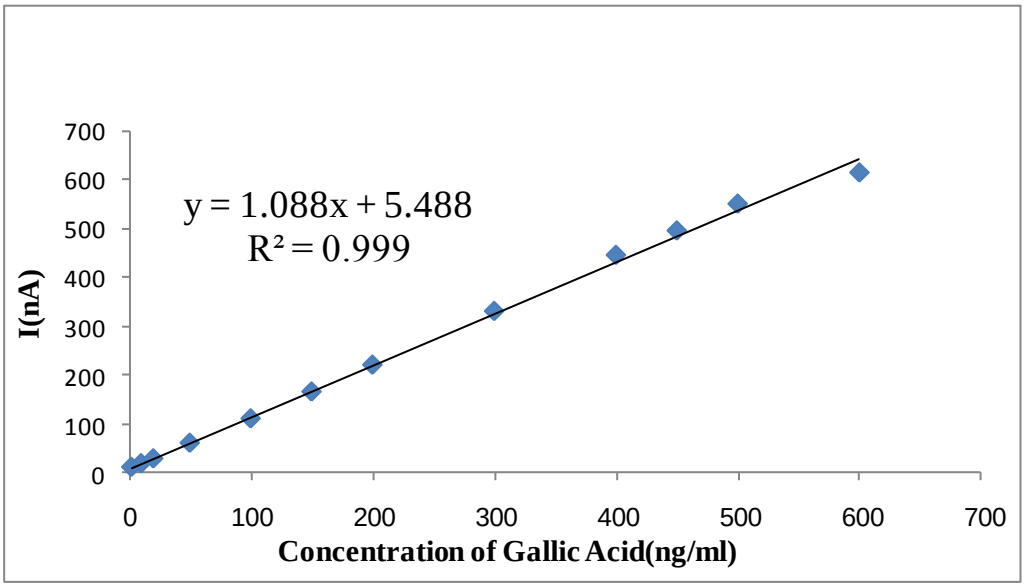


Figure.6. Calibration curve for determination of GA from 0.1 to 600 ng/ml. Conditions:, accumulation potential, +50 mV; accumulation time, 100 s; scan rate 80 mV/s.

3.5. Interferences of coexistence species

The influence of various foreign species on the determination of GA was examined. The tolerable limit of the foreign species was taken when the relative error was not greater than ±5% in the recovery. It was found that the tolerable concentration ratios with respect to 1×10^{-7} M GA were as species shown in table 1. In addition n the presence of 50 fold of potassium sulfide and uric acid, 20 fold of ascorbic acid, 10 fold of glucose, barbituric acid, the determination was not interfered. As to surfactants, TritonX-100, Cetyltrimethylammonium bromide (CTAB), sodium dodecylsulfat (SDS), was tested. In present of 1000 fold of SDS, the peak current of GA kept almost unchanged. However the addition of 10 fold TritonX-100 made the reduction peak of 1×10^{-7}M GA decreased by 9.6%. This is due to the competitive adsorption of GA on HMDE surface.

Table 1. Interference study for the determination of GA under optimum conditions.

Species	Tolerance limit(W_{ion}/W_{GA})
Na^{+},ClO_3^{-},Cl^{-},Li^{+},BrO_3^{-},IO_3^{-},I^{-},$H_2PO_4^{-}$ Fe^{+2},Tartarate,CH_3OH,Ethanol,PO_4^{-3},K^{+},Mg^{+2},CH_3COO^{-}	1000
Ni^{+2},$C_2O_4^{-2}$,SO_4^{-2},SCN^{-},NO_3^{-},CN^{-},	500
Sn^{+2},Zn^{+2}	200
HPO_4^{-2}	100
Pb^{+2},Fe^{+3},Cd^{+2},Cu^{+2},CO_3^{-2}	50

3. 6. Application of the proposed method for real samples

To evaluate the applicability of he proposed method, it was applied to the determination of gallic aid in plasma, urine and tea samples (fig. 7). The results of recommended procedure, with a standard addition method are summarized in tables 2. Satisfactory results are obtained by the proposed method.

Table 2. Determination of GA in real samples (n=3).

Sample	GA added (ng/ml)	GA found (ng/ml)	R.S.D.(%)	Recovery(%)
Plasma	0.0	7.73	0.15	-
Plasma	30	36.93	0.95	97.33
Plasma	50	56.04	0.75	96.62
Urine	0.0	11.56	0.21	-
Urine	30	41.16	0.65	98.66
Urine	50	62.04	0.82	100.96
Tea	0.0	32.468	0.29	-
Tea	30	61.51	0.22	97.20
Tea	50	82.46	0.21	100.06

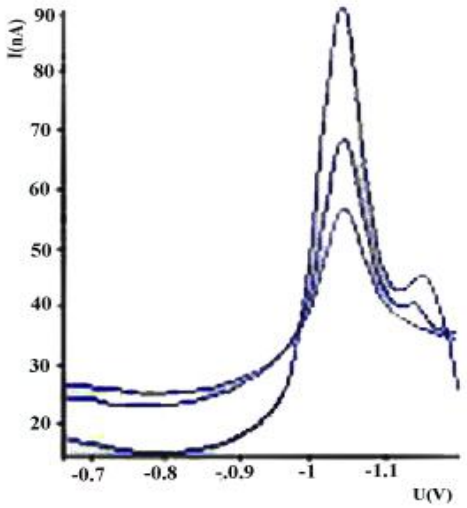


Figure.**7**. Voltammograms related to the determination of GA in tea sample. Conditions; optimal instrumental and concentration conditions.

4. CONCLSIONS

The paper showed that adsorptive stripping voltammetry is a useful method for the highly sensitive and selective determination of GA. The proposed procedure is simple and inexpensive, also is more sensitive than existing methods for determination of GA. it was found that GA is unstable in plasms and urine at room temperature, so its stability was enhanced by adding 1 ml sulfuric acid immidiately to the collected plasma samples. The results of this study are more sensitive [21-23], selective[13,14], specific[17,18,24] and short time analysis [19]. The method has been successfully apllied to the determination of GA in tea, urine and plsma serum.

ACKNOWLEDGEMENTS
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