ELECTROCHEMICAL STUDY OF ELLAGIC ACID (CANCER CHEMO PREVENTIVE AGENT) IN WALNUTS & ITS FORMULATION

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ABSTRACT

DC and DP polarographic methods have been developed for the qualitative as well as quantitative analysis of ellagic acid in plant products *(i.e.* walnuts*)* and market formulation (Jelly Crystals - REX^{TM}). Ellagic acid produces a well defined polarographic wave/peak in pyridine hydrochloride at pH 6.8± 0.1 with $E_{1/2}$/Ep = -1.298 V vs SCE.

The wave/peak height is found to be proportional to the concentration of ellagic acid. The developed procedure was used for the analysis of ellagic acid in extract of walnuts (*Juglans regia* Linn.) and pharmaceutical formulation. Statistical treatment of the observed polarographic data revealed high accuracy and good precision of determination.

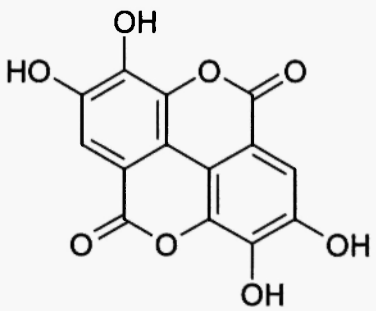
The work has been supplemented by FTIR screening of the sample. Ellagic acid exhibits absorption bands at about 1111 cm^{-1}, 1195 cm^{-1}, 1224 cm^{-1}, 1263 cm^{-1}, 1321 cm^{-1}, 1472 cm^{-1}, 1715 cm^{-1}, 3473 cm^{-1} and 3520 cm^{-1}. The percentage of ellagic acid in market formulation was determined by the developed method, which is in close agreement with that reported by the manufacturers.

Keywords: Ellagic acid, Walnuts, Formulation, DCP, DPP Analysis

INTRODUCTION

Ellagic acid ($C_{14}H_6O_8$) is a naturally occurring polyphenolic found in forty six different fruits and nuts such as raspberries, strawberries, blackberries and walnuts /1/. Ellagic acid behaves as a strong antioxidant /2/. It is also a potent anticarcinogen and has the ability to inhibit mutation /3/ within a cell's DNA. It is also considered be a cancer inhibitor, which has the ability to cause apoptosis or normal cell death in cancer cells /4/. It has antibacterial and antiviral properties. It acts as a scavenger to bind cancer-causing chemicals, making them inactive. It inhibits the ability of other chemicals to cause mutations in bacteria.

Walnuts (*Juglans regia* Linn.) are an excellent source of ellagic acid which has been shown to provide many health benefits. Ellagic acid has been determined earlier in plant products using HPTLC /5/, HPLC /6,7/, gas chromatography and other spectroscopic techniques /8/. The literature records that the polarographic and voltammetric methods have been widely used for the analysis of organic compounds in samples of natural origin /9,10/. However, the voltammetric methods have not been tried for the analysis of ellagic acid. The authors have therefore developed DCP and DPP methods for the analysis of ellagic acid in samples of plant origin and in pharmaceutical/market formulation. The work has been supplemented by FTIR spectral studies, the results of which have been reported in this paper.



Ellagic acid

EXPERIMENTAL

Chemicals and Reagents

The chemicals used were of Anal-R grade, whereas the ellagic acid was from Himedia Laboratories Pvt. Ltd. Mumbai. (5mM) Ellagic acid solution was prepared by dissolving the requisite amount in pyridine.

Apparatus

The DCP and DPP studies were carried out on an Elico (India) micro-processor based polargraphic analyzer, model CL-362. The polarographic cell consisted of an electrode assembly having a dropping mercury electrode, a coiled platinum wire electrode and a saturated calomel electrode. The capillary characteristics of the DME had a $m^{2/3}$ $t^{1/6}$ value of 2.5$mg^{2/3}$ $sec^{-1/2}$ at 60 cm effective height of mercury column. A Systronics digital μpH meter model-361 was used for the pH measurements. The UV studies were carried out on an Elico (India) SL 164 double beam UV-Vis spectrophotometer. The FTIR studies were carried out on a Shimadzu (Japan) FTIR-8400S model.

Extraction of Ellagic Acid from Walnuts (*Juglans regia* Linn.)

15g of ground walnuts were weighed into a 50ml polyethylene centrifuge tube with 20ml of methanol. The sample was extracted for 1h at room temperature on an orbital shaker operated at 400 rpm and then centrifuged at 6000 rpm, and the supernatant was immediately analyzed. Walnuts were extracted by methanol at 100°C for 24h. The extract was then evaporated to dryness and hydrolyzed in 2N trifluoroacetic acid in methanol at 100°C for 2h.

Polarographic Determination of Ellagic Acid

A known concentration of ellagic acid was taken in a polarographic cell. The volume of analyte was made up to 50ml with pyridene. The pH was adjusted to 6.8 ± 0.1 with dilute solution of NaOH/ HCl. A polarogram was then recorded.

Ellagic acid produces a well-defined polarographic response in pyridine hydrochloride solution. The wave height/peak height of the polarogram was

found to be proportional to ellagic acid concentration. Calibration curve was obtained by taking different known concentration of ellagic acid in pyridine hydrochloride supporting electrolyte under identical experimental conditions as discussed above and recording the polarograms and plotting i_d/i_p vs ellagic acid concentration curve.

Determination of Ellagic Acid in Walnuts Extract

For the determination of ellagic acid in walnuts extract, a known weight (5g) of extract was dissolved in 50ml of pyridine. The pH of the test solution was adjusted to 6.8 ± 0.1 with dil HCl/ NaOH solution and the polarogram was recorded. The amount of ellagic acid present in the extract was determined with the help of calibration curve and standard addition method.

Determination of Ellagic Acid in Market Formulation (Jelly Crystals - REX™)

For the determination of ellagic acid in market formulation, a known weight (5g) of formulation was dissolved in 50ml of pyridine. The pH of the test solution was adjusted to 6.8 ± 0.1 with dil. HCl/NaOH solution and its polarogram was recorded as discussed earlier.

RESULTS AND DISCUSSION

The direct current polarogram (DCP) and differential pulse polarogram (DPP) Figs. 1 and 2 of the authentic sample solution of ellagic acid in Pyridine hydrochloride at pH 6.8 ± 0.1 produced one well defined polarographic wave/peak with $E_{1/2}/E_p$ = -1.298 V vs SCE, indicating the presence of ellagic acid in the sample.

To ascertain whether the wave/peak is due to ellagic acid, a known quantity of standard solution of ellagic acid was added to the analyte and polarogram was recorded under above experimental conditions. An increase in wave height/peak height of the poloragram due to ellagic acid was observed without any change in half wave/peak potential, thus, confirming the presence of ellagic acid in the sample. To standardize the developed polarographic procedure for the quantitative and qualitative analysis of

ellagic acid, some experimental sets with varying concentration of ellagic acid were prepared and their polarograms were recorded, under experimental conditions discussed above. The results indicated no change in the $E_{1/2}/E_p$ value of the ellagic acid. It was also observed that the concentration of ellagic acid is directly proportional to its wave/peak height, thus, reconfirming the possibility of an accurate qualitative and quantitative determination of the ellagic acid in the sample.

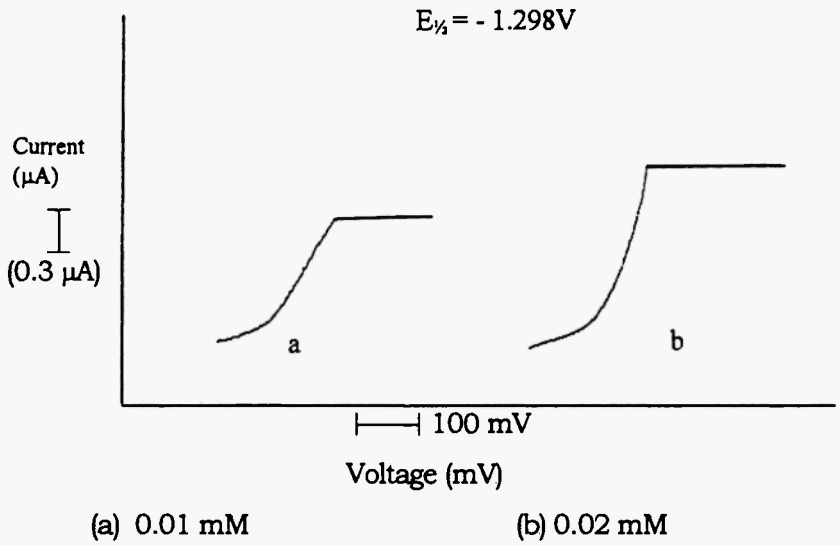


Fig. 1: Direct current polarograms of ellagic acid in pyridine hydrochloride at pH 6.8 ± 0.1.

Figs. 3a and 4a show the DC and DP polarograms for ellagic acid content in walnuts, whereas Fig. 3b and 4b show the DCP and DPP for the ellagic acid content in Jelly Crystals - REX™, a market formulation.

Each of these figures clearly shows a well defined polarographic response with $E_{1/2}/E_p$ = -1.298 V vs SCE for the presence of ellagic acid in the sample. A method of external spiking was used for the analysis of ellagic acid in the extracted samples from walnuts and Jelly Crystals - REX™.

A small change in the $E_{1/2}/E_p$ value of ellagic acid was observed in the polarogram of extracted sample of walnuts as compared to the $E_{1/2}/E_p$ value observed with solution of authentic sample, which may be due to matrix

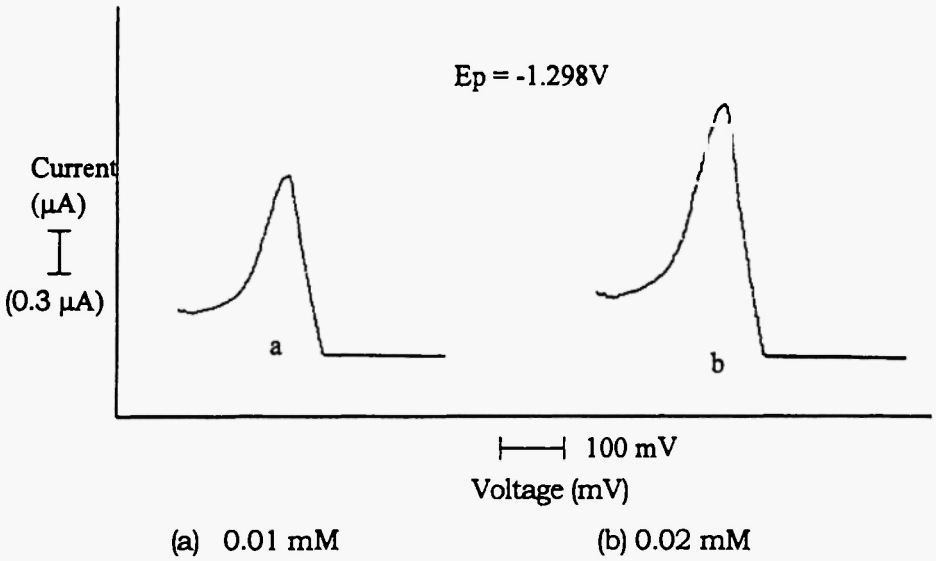
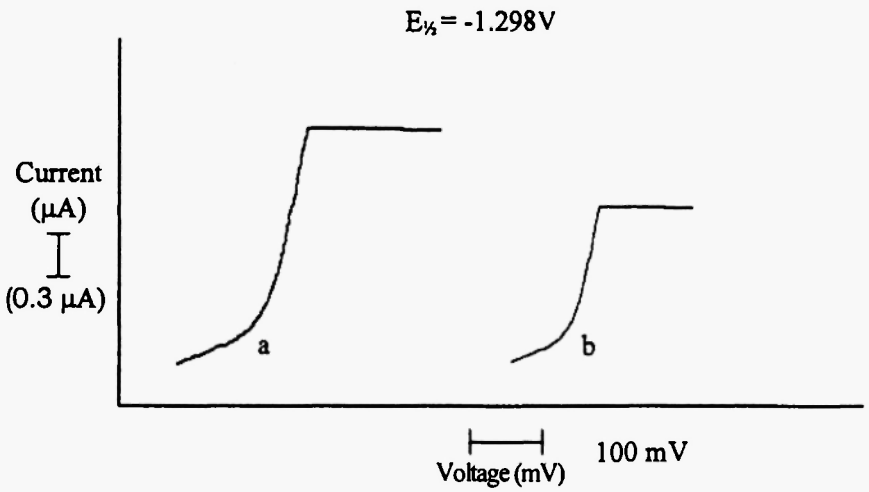


Fig. 2: Differential pulse polarograms of ellagic acid in pyridine hydrochloride at pH 6.8 ± 0.1.



(a) Walnuts extract (b) Jelly Crystals - REX(market formulation)

Fig. 3: Direct current polarograms of extracted ellagic acid in pyridine hydrochloride at pH 6.8 ± 0.1.

Table-1

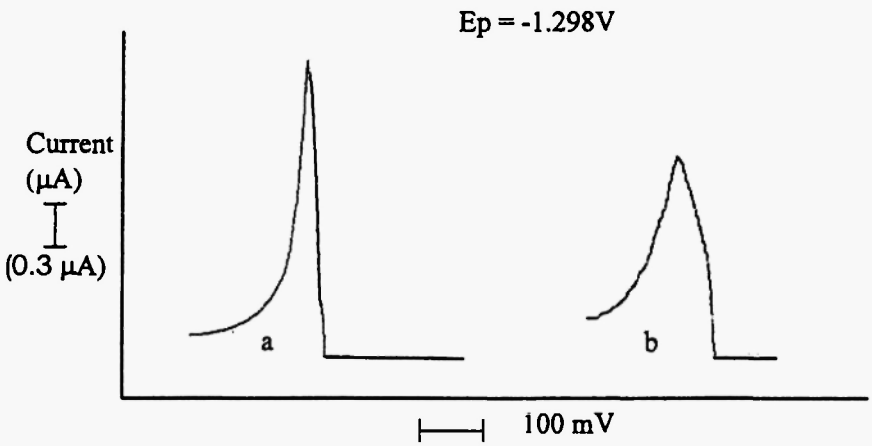
Ellagic acid[a] in walnuts & Jelly Crystals - REX™

Sample	Added (mg)	Found (mg/g)	%R	S.D
Walnuts Extract	- 0.5 -	0.587 - 1.080	99.90	0.002
Jelly Crystals (Market formulation)	- 0.1 -	0.161 - 0.260	99.62	0.001

a = Results are average of five determinations

R% = Recovery Percent

S.D. = Standard deviation.



(a) Walnuts extract (b) Jelly Crystals - REX(market formulation)

Fig. 4: Differential pulse polarograms of extracted Ellagic acid in Pyridine hydrochloride at pH 6.8 ± 0.1.

effect. As such, to determine its concentration in the extracted samples, the method of spiking was used which not only served the purpose of quantitative analysis of ellagic acid content in the samples but also helped to avoid the problem due to matrix effect. The validity of the developed polarographic method for the ellagic acid analysis in the natural origin samples was proved by the percentage recovery and standard deviation of the data (Table 1). The percentage recovery was always found to be above 99.5% and the standard deviation never exceeded 0.03, thus confirming the reliability of the analysis. On the basis of the observed polarographic data the concentration of ellagic acid in walnuts and Jelly Crystals - REX^{TM} are found to be 587μg/g and 161μg/g respectively.

The work was supplemented by FTIR screening of the extracted samples for its Ellagic acid content. Ellagic acid exhibits absorption bands at 1111 cm^{-1}, 1195 cm^{-1}, 1224 cm^{-1}, 1263 cm^{-1}, 1321 cm^{-1}, 1472 cm^{-1}, 1715 cm^{-1}, 3473 cm^{-1} and 3520 cm^{-1} corresponding to C–O–C group stretching, C–C–O group stretching, C–O stretching (alcohol), C=C ring stretching, C=O group stretching (lactones), O–H stretching (Intramolecular hydrogen bonded OH group) and O–H stretching (free O – H group) respectively. Similar FTIR signals were also observed for authentic ellagic acid sample, thus confirming the presence of ellagic acid in the extracted sample from walnuts.

The amount of ellagic acid in market formulation was determined by the developed method, which is in close agreement with that reported by the manufacturers.

CONCLUSION

On the basis of the observed data and ongoing discussion it may be concluded that the developed polarographic procedure could be successfully used for the accurate analysis of ellagic acid in plant extract and industrial samples.

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