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DETERMINATION OF MONOVALENT COPPER IN DIFFERENT HERBS AND THEIR MIXTURE USING DIFFERENTIAL PULSE POLAROGRAPHY

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ABSTRACT

Monovalent copper is determined in different herbs and their mixture using differential pulse polarography (DPP) and standard addition method is employed. Selected herbs are *Nigella sativa* (Kalonji), *Trigonella foenum-graceum* (Fenugreek) and *Linseeds* (Alsi), in the form of mixture they are taken in ratio 2:1:1 respectively. For the extraction of samples aqueous as well as acidic media were used. In the acidic medium the average values of concentrations for individual herbs and also in the mixture are greater as compare to the aqueous medium. The average value of copper in the mixture is found to be moderate in both media as compare to the individual herbs. These values also comparable to the reported tolerance limit. Polarograms were recorded in the differential pulse mode with multimode electrode system of Metrohm A. G. model 746 VA Trace analyzer connected with data based software. Copper is required to the body in very little amount but this little quantity is very important and essential. The quantitation is made by standard addition method and linearity is calculated by linear regression line. Linearity of differential pulse polarography is checked by the calibration curves.

Keywords: Monovalent copper, differential pulse polarography, *Trigonella foenum graceum, Nigella sativa*.

INTRODUCTION

Naturally occurring alternative medicine could help to solve many of the common as well as peculiar medicinal problems facing the people (Vohora and Dandiya, 1992; Takri and Dameh, 1998). There are many herbs like *Nigella sativa* commonly known as Kalonji, *Linseeds* commonly known as Alsi and *Trigonella Foenum-graceam* (fenugreek) commonly known Methi used for the treatment of different diseases such as cancer, diabetes, arthritis, asthma, allergies etc. (Miller, 1998). These selected herbs have over one hundred different chemical constituents most of them show antioxidant ability (Al-Yahya, 1981; Bose *et al*., 1981). They have many pharmacological actions and act individually as well as in the combined form or a mixture. Seeds of Nigella sativa were used in the indigenous system of medicine (Salomi and Nair, 1992) and act as a anti-phlegmatic, stimulant, carminative, diuretic, expectorant, anti-fertility, stomach and liver tonic (Burits and Bucar, 2000). Linseeds are used for the treatment of heart disease, cancer, diabetes, arthritis, asthma, syndrome, allergies, inflammatory tissues conditions, skin conditions, vitality, calmness under stress (www.vedamsbooks, 2000). Fenugreek is extensively used both internally and externally on account of their cooling properties. It is applied to skin as cosmetic in the form of powder. The regular consumption is beneficial in the management of diabetes (Fatima *et al*., 2004).

The role of elements in health and disease is now an established fact (Siddiqi and Khan, 1990 and Hewamanna *et al*., 2004). Trace elements are crucial to virtually all biochemical and physiological processes in plant, animal and human beings (Nergiz and Otle, 1992). Of these, iron, zinc, cobalt, manganese, nickel, copper, chromium and molybdenum are now thought to be essential for animal life (Cheblowski and Coleman, 1976). Herbs are also the source of Fe, Cu, Zn, Cr, Mn, Co, Mg, Na, Ca, K and Se which required in small amounts by the body (Shahito *et al.*, 2002 and Fatima *et al.*, 2005[a]). The main action of these elements is to act as essential cofactors in various enzymatic functions. Most of the trace elements like iron and copper play active role in their lower oxidation states because maximum absorption of them take place in the body in such states (Iffat and Fatima, 2006). Copper is the third most abundant element in human body, following iron and zinc (Bratter and Schramel, 1980). Copper shifts between the monovalent (cuprous) and the divalent (cupric) forms in the body. The ability of copper to easily accept and donate electrons explains its important role in redox reactions and scavenging of free radicals (Cowan, 1997). Though the majority of body's copper is in the divalent form but the monovalent form is also a critical functional component of a number of essential enzymes, known as cuproenzymes. The copper dependent enzyme, cytochrome c oxidase, plays a critical role in cellular energy production. Two copper containing enzymes, ceruloplassmin, ferroxidase I and ferrioxidase II have the capacity to oxidize ferrous iron to ferric iron, the form of iron that can be loaded onto the protein transferring for transport to the site of red blood cell formation. Iron mobilization from storage sites is impaired in case of copper deficiency (Pittand and Martell, 1980). The determination of inorganic elements in biological and environmental materials at trace and ultratrace levels has now become a major aspect. For this reason several analytical techniques such as atomic absorption spectrophotometry

and spectrophotometry (Siong *et al.*, 1989) have been developed that are capable of determining the elements reliably at trace levels. But now numerous variants of electroanalytical methods for example differential pulse anodic stripping voltammetry (DPASV), cathodic stripping voltammetry (CSV), differential pulse polarography (DPP) etc., are being used for trace analysis of metals. These techniques are now being used more in determination of trace elements in biological and environmental materials owing to their simplicity, improved selectivity and high sensitivity. The bioavailability of metals from selected herbs was investigated and it was concluded that they were better utilized and play role in health (Fatima *et al.*, 2005[b]).

In the present work monovalent copper is determined in selected herbs *Nigella sativa, Trigonella foenum-graceum* and *Linseeds* as well as in their mixture using differential pulse polarography. Standard addition method is employed for this study. The aim of this study is to determine the amount of monovalent copper supplied by these herbs and their mixture and also to establish polarographic method for the analysis of monovalent copper. Our goal is to determine the role of these selected herbs for human health.

MATERIALS AND METHODS

Sampling and sample preparation

The samples of selected herbs *Nigella sativa, Trigonella foenum-graceum* and *Linseeds* were purchased from the local market. They were cleaned and dried at 40 ± 5^0C. Accurately weighed amount of sample (about 4gms) and for extraction of the samples aqueous as well as acidic media were used. For aqueous medium the samples were taken in about 100ml ml deionized water at 45^0C for about 45 minutes and for acidic medium the samples were soaked in about 100ml of 0.1M HCl for 2hours. Then filtered them with Whatman 42, aqueous filtrate was then made upto 100ml with distilled water and acidic filtrate made upto 100ml with 0.1M HCl. Mixture of herbs also treated in the similar manner using aqueous and acidic media, taking accurately weighed amount about 4gms *Nigella sativa*, 2gms *Trigonella foenum-graceum* and 2gms *Linseeds*, showing ratio 4:1:1 with final volume of filtrate made upto 100ml.

Preparation of standard solution

All chemicals were of analytical reagent grade used throughout the analysis supplied by Sigma or Merck. Stock standard solutions of the $CuCl_2$ was prepared first, about 0.1gm was dissolved in 25ml of deionized water, few drops of concentrated nitric acid was added, diluted to 100ml deionized water and mixed thoroughly (1000ppm). Working standard solution was prepared by taking 25ml of stock solution in 100ml of volumetric flask and volume made upto mark with deionized water. 30% HCl was prepared by diluting 40mL of concentrated HCl upto 50mL with deionized water.

Instrumentation

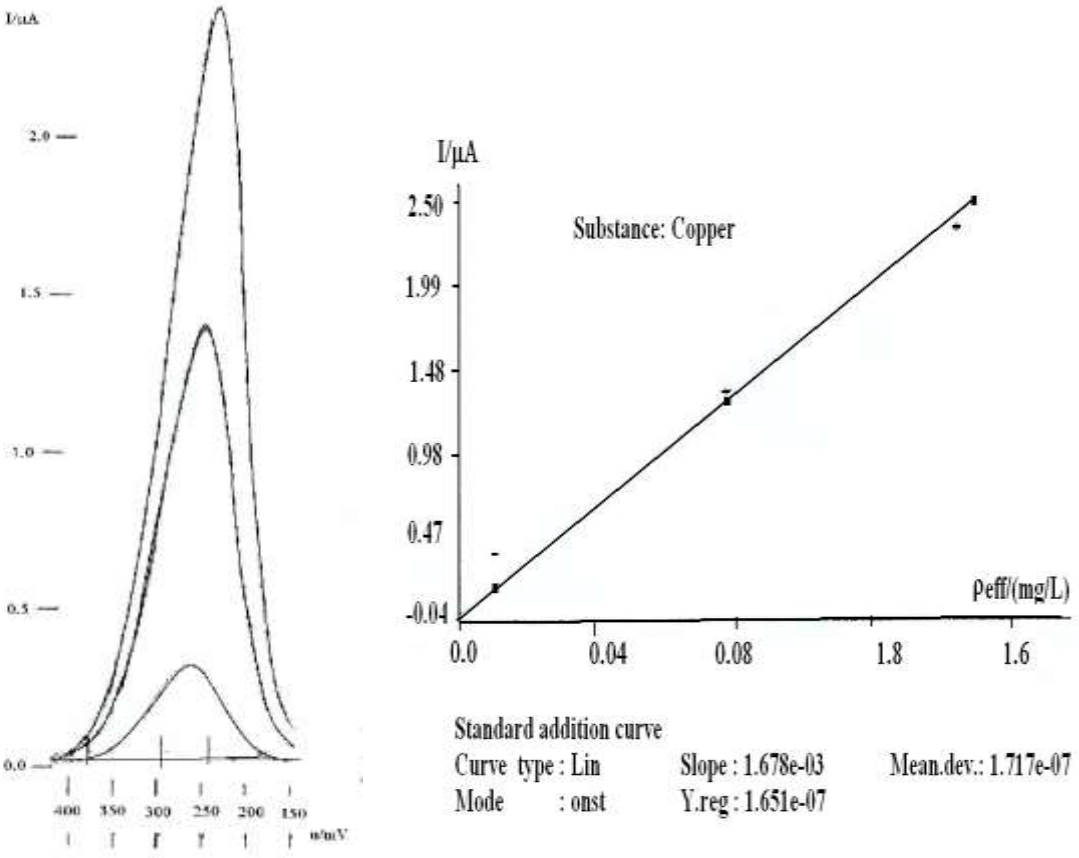
Polarograms were recorded in the differential pulse mode with multimode electrode system of Metrohm A. G. model 746 VA Trace analyzer connected with data based software. The analyzer was consisting a pear shaped glass cell with three electrodes, a hanging mercury drop electrode (HMDE) as the working electrode, a thin platinum(Pt) rod as the auxiliary electrode and an Ag | AgCl electrode as reference electrode. Other parameters were set as; stirring electrode: 2000/min, purge time: 300sec, deposition time: 10min, pulse amplitude: 50mV, start potential: -450mV, end potential: 0mV, peak potential: -284mV, voltage step time: 0.2sec and sweep rate: 20mV/sec.

Polarogramic analysis

A 10ml of herbal extract was taken in the glass cell and then 5ml of 30% HCl act as supporting electrolyte was added into it. The solution was deaerated with pure (99.99%) nitrogen for about 10 minutes. The inert atmosphere in the polarographic cell was maintained during the determination by a blanket of nitrogen. The standard addition method was used for the evaluation of the copper content. All the herbs individually as well as in the form of mixture were analyzed in the same manner. A blank of the reagents was also checked for comparison.

RESULTS AND DISCUSSION

The selection criterion of these herbs is their uses in the treatment of different diseases. The mixture of them in the given ratio (metioned in previous section) is taken by the most of the diabetic patients in Pakistan to control sugar level in the body. Copper is required to the body in very little amount but this little quantity is very important and essential. Normal concentration of copper present in the human tissues is 2ug/gm and tolerance limit is 80-110ug/gm (Franklin *et al.*, 1983).



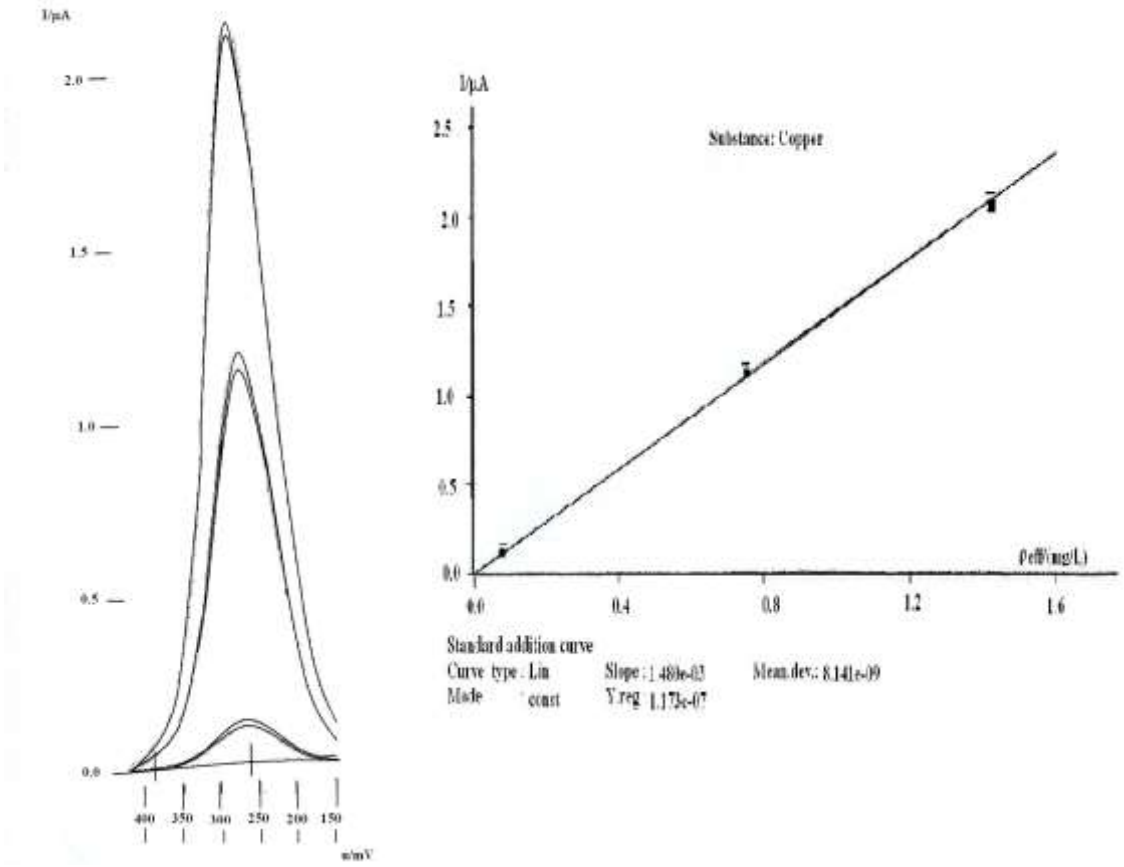
Substance: Copper U = -296mV I =23.437nA

Fig. 1 Differential pulse polarogram and standard addition curve for *Nigella sativa*

In the present study the monovalent copper is determined in herbs and their mixture by differential pulse polarography. Spectroscopic methods can only determine the total concentrations of metals but with polarography it is additionally possible to differentiate between the different oxidation states of metal. The possibility of simultaneous determination of several metals, good precision, inherent accuracy and low instrumental cost in comparison with other trace analytical methods are the basic reasons to choose this technique for analysis. Five samples of each herb and also five sets of mixture were treated. The polarograms of *Nigella sativa*, *Trigonella foenum-graceum*, *Linseeds* and their mixture (2:1:1) for one set of aqueous medium are presented in **Fig. 1-4** respectively. Peak evaluation is carried out according to the given program feed in computer based software and mathematical analysis is done itself by peak potential and peak height. The quantitation is made by standard addition method and linearity is calculated by linear regression line. Linearity of differential pulse polarography is checked by the calibration curves of concentrations against peak heights for *Nigella sativa*, *Trigonella foenum- graceum*, *Linseeds* and their mixture (2:1:1) for one set of aqueous medium are presented in **Fig. 1-4** respectively.

Table 1. Concentration of monovalent copper in different herbs and their mixture using aqueous medium.

S. No.	***Nigella sativa* ug/gm**	***Trigonella foenum graceum* ug/gm**	***Linseeds* ug/gm**	**Mixture 2:1:1 ug/gm**
1	142.89	113.90	44.87	74.67
2	148.95	120.54	36.51	76.92
3	143.99	119.99	42.34	71.55
4	148.55	114.50	41.95	76.48
5	151.75	122.95	35.23	80.01
Mean value	**147.23**	**118.38**	**40.18**	**75.93**



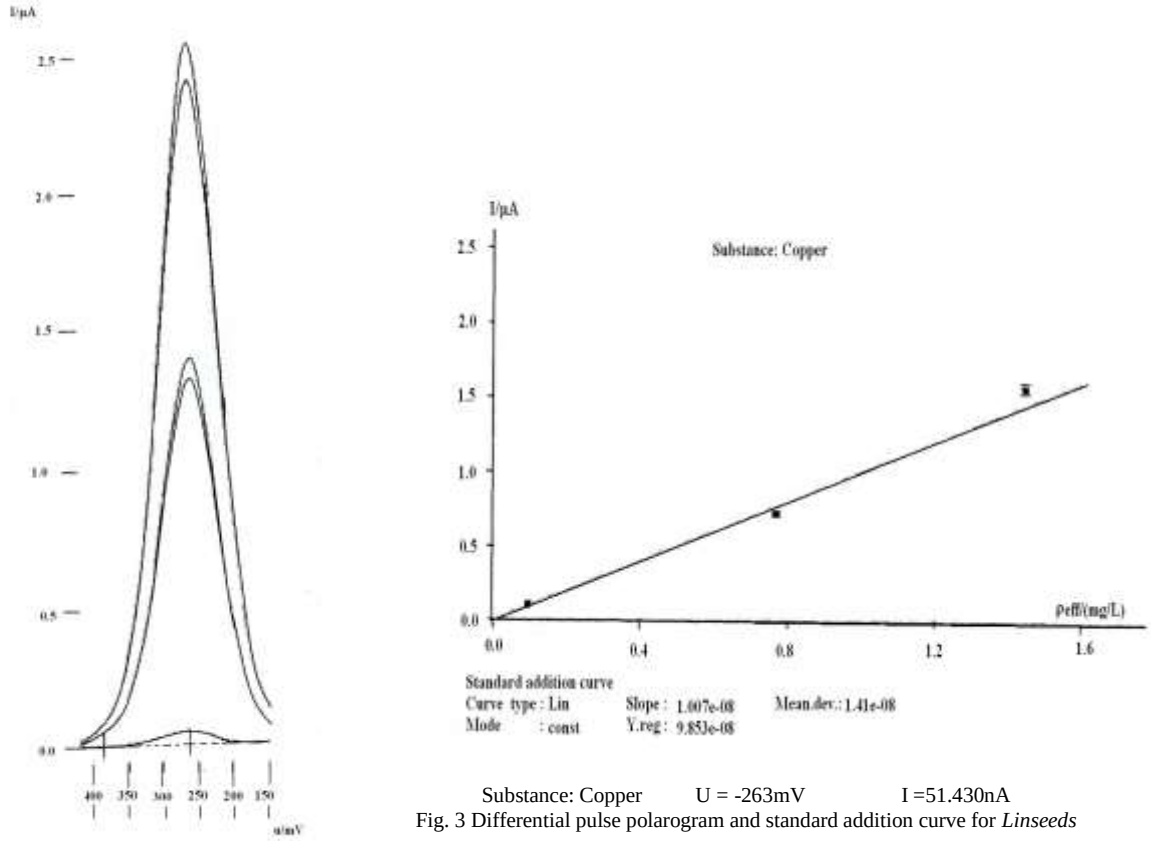
Substance: Copper U = -261mV I =122.411nA

Fig. 2 Differential pulse polarogram and standard addition curve for *Trigonella foenum-graceum.*

Franklin *et al.*(1983) was also determined the monovalent copper using polarography in which the peak potential was observed at -270mV that is comparable to our peak potential showing in **Fig. 1-4**. Concentrations of monovalent copper in all five samples of different herbs and their mixtures are given in table 1 and 2 for aqueous and acidic media respectively. In the acidic medium the average values of concentrations for individual herbs and also in the mixture are greater as compare to the aqueous medium. It means that in the acidic conditions, extraction of copper is higher than in neutral conditions. It is also interesting to note that in the form of mixture the average value is moderate in both media as compare to the individual herbs, which is comparable to the reported tolerance limit. The data for the individual herbs is shown that aqueous extract of *Trigonella foenum-graceum* is better as copper supplement as compare to others. At the end, it is concluded that selected mixture of herbs is the safe and effective way to use as copper supplement and treatment of other diseases.

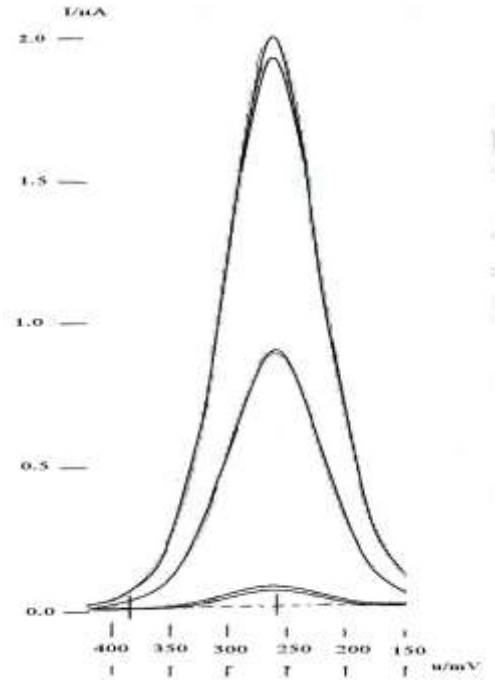
Table 2. Concentration of monovalent copper in different herbs and their mixture using acidic medium.

S. No.	***Nigella sativa* ug/gm**	***Trigonella foenum graceum* ug/gm**	***Linseeds* ug/gm**	**Mixture 2:1:1 ug/gm**
1	185.98	152.04	55.67	100.42
2	191.09	149.67	53.90	102.98
3	192.95	143.98	61.97	108.54
4	186.43	146.63	58.98	106.93
5	194.75	147.99	56.34	105.99
Mean value	**190.24**	**148.06**	**57.37**	**104.97**



Substance: Copper U = -263mV I =51.430nA

Fig. 3 Differential pulse polarogram and standard addition curve for *Linseeds*



Substance: Copper U = -260mV I =67.635nA

Fig. 4 Differential pulse polarogram and standard addition curve for mixture of *Nigella sativa, Trigonella foenum-graceu and Linseeds* (2:1:1)

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