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RESEARCH ARTICLE

Fast and Cost Effective Electrochemical method for Ascorbic Acid in Pharmaceutical labs

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Abstract

A fast and inexpensive analysis is a prime requirement of all laboratories. This study establishes voltammetric method for quantitative analysis of vitamin-C (Ascorbic Acid) in different pharmaceutical products. Oral tablets, drops, syrups and capsule are analyzed for vitamin-C assay in drugs. Potassium chloride of 0.3M is chosen, as supporting electrolytes. Optimum scan rate is found $100mVs^{-1}$. Glassy carbon electrode is chosen as indicator electrode. Half wave potential is 350mV as average. Average peak potential is 400mV. A sensitivity of $21.93mA.M^{-1}$ is obtained with detection limit of 0.001mM. Expected interference (due to excepients) is examined. Shifting of half wave potential is observed in case of syrup, capsule and core tablet as matrix interference. While no such effect of shifting in case of in injeactables. System is found to be non reversible. Spiking of samples with standards overcome the problem of having shifting of half wave potential.

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INTRODUCTION

Pharmaceutical labs are always busy and analysts are always eager for fast methods with quality analysis. Moreover owners of pharmaceutical industry and labs are more worried about the cost. This study has been established to satisfy both analyst and their bosses.

Antioxidants are popular in medicine and almost pharmaceutical industries produce antioxidant products. Ascorbic acid is one of the common medicinal antioxidant. Ascorbic acid is more commonly known as vitamin C that is one of the essential nutrients. Recommendation of daily intake for children ages (1-3 years) is 15mg/day, (14-18 years) ages females is 65 mg/day, old females is 75mg/day and (14-18years) ages males is 75mg/day and old males is 90mg/day [Julia R. Esch et al.,2010]. Deficiency of vitamin causes number of detrimental health effects like scurvy that is most serious and well known diseases. Therefore daily intake of vitamin C is so important. Being powerful antioxidant vitamin C fights against free radical induce diseases [Aurelia Magdalena Pisoschi et al., 2008]. It has significant role in production of collagen, a protein needed in the development and maintenance of cartilage, joint linings, blood vessels, skin, bones, teeth and gums. The beneficial effects of vitamin C are not restricted to its anti-scorbutic activity as there are now evidences to show that it is protective against neurodegeneration and may ameliorate some cardiovascular diseases and also have anti cancer affects [Li, Y. and Schellhorn, H.E. 2007].

A variety of methods are being used for the qualitative and quantitative determination of ascorbic acid such as HPLC [E. J. Oliveira et al., 2001; M. A. Kall et al., 1999; H. Iwase et al., 1998], spectrophotometric methods [Kubilay Guclu et al., 2005] and also a classical (convectional voltammetric method) using Potassium Iodate [Balan D et al., 2005], Bromate etc. [Matei N et al., 2004]. Voltammetry is a popular method that applies to ascorbic acid determination in real samples. Voltammetry has low detection limit comparative to other expensive techniques. Little or almost no sample preparation is required. This technique is fast as well as easier. Voltammetry is an attractive alternative to the titrimetric or other instrumental methods because of cost effectiveness, simplicity, less

consumable requirement. This technique can be operated by less qualified persons and it is also less laborious [Aurelia Magdalena Pisoschi et al. 2008]

Out of voltammetry, cyclic voltammetry is also one of the most popular voltammetric methods for qualitative and quantitative analysis. It is also well reported for determination of ascorbic acid in real samples [Aurelia Magdalena Pisoschi et al. 2008; Holle and Santos 2010; Julia R. Esch et al., 2010].

The aim of this study is to investigate improved method for determination of ascorbic acid in pharmaceutical products by employing electrochemical analysis such as cyclic voltammetry, amperometry etc. The reported data in literature regarding the determination of ascorbic acid by this method are very scarce [Koh Sing Ngai et. al., 2013; Siamak Gheibi et al., 2013]. The developed method was applied to the determination of ascorbic acid in different types of pharmaceutical products containing ascorbic acid.

Experimental Method

Reagents and apparatus

The chemical are used throughout the study are of analytical grade such as Potassium Chloride KCl (MERCK^{TM}), Lithium per Chlorate $LiClO_4$ (SHARLU^{TM}), and Tetra butyl almunium per chlorate $TBAClO_4$ (TCI^{TM}), Dimethyl Sulfoxide DMSO (LAB-SCAN^{TM}) and Ascorbic acid (BIO-BASIC^{TM}). Deionized distilled water is used throughout. Different pharmaceutical products like core tablets, syrups and capsules are collected from local pharmacies of Karachi, Pakistan during June and July 2013. Intentionally, commercial names of pharmaceutical products are not mentioned.

Cyclic voltammetry is carried out at an electrochemical workstation (CH-Instrument model 760-D); the quantitative work was done by calibration curve method and standard addition method. Three electrodes system are used, comprising of Glassy Carbon (GC) as working electrode, Platinum (Pt) as counter electrode and Saturated Calomel Electrode (SCE) as reference electrode.

Supporting electrolyte such as $LiClO_4$ and KCl are prepared in deionized distilled water and $TBACLO_4$ is prepared in DMSO. The stock solution (5 mmole.L^{-1} L-Ascorbic Acid) is prepared in distilled water. From the stock solution the working standards of 0.1, 0.2, 0.3, 0.4 and 0.5 mmol.L^{-1} vitamin-C are diluted in (0.3 moleL^{-1}) KCl. Finally, solutions are run at voltammetry.

Samples Preparation

Since core tablets, syrups and capsules are being analyzed in this study. Therefore, different sample pretreatment are carried out for different form of drugs. Detail of such pretreatment is given below.

CORE TABLET

Pre-weighed core tablet is dissolved and finally make up with supporting electrolyte (0.3M KCl) in 100ml volumetric flask. Then this sample flask is placed in ultrasonic bath for 10 minutes and then filtered by using 0.45µm size Nylon filter paper (Millipore^{TM}) that is coupled with air pump for vacuumed suction. Usually, 100µl of sample is run with employing standard addition method.

SYRUP

A stock solution of vitamin-C syrup is prepared in 50ml volumetric flask by transferring 1.84gm of syrup. Then further (1:10) diluted sample in 25ml volumetric flask is prepared by dilution with supporting electrolyte.

CAPSULE

Single capsule is dissolved in water with continuous heating at about 50^{o}C. After dissolution, this solution is filtered through 0.45µm (Millipore^{TM}) Nylon filter paper. Finally make up the volume with supporting electrolyte in 500ml volumetric flask. A 500µl aliquot of diluted sample is run.

Result and discussion

Quantitative chemical analysis is always depending upon chemical or physical changes under some conditions or surrounding. These conditions must be optimized for having best results. To avoid unnecessary oxidation, analysis runs are carried out for Vitamin-C with purging of (5N) nitrogen. Selection of suitable supporting electrolyte (that encounters migration current in the cell) has been done. Supporting electrolyte minimizes migration current and IR-drop. In this study potassium chloride (KCl), lithium perchlorate ($LiClO_4$) and tetra butyl ammonium per chlorate ($TBAClO_4$) have been examined as supporting electrolyte (Fig. 1). Out of which KCl gives optimum diffusion current. Tetra-butyl ammonium perchlorate is showing very low or almost no signal for reaction. This means $TBAClO_4$ is completely failed to overcome migration current for vitamin C. Lithium perchlorate is also employed as supporting electrolyte that results broader peak (Fig. 1). Different concentrations of KCl (as a supporting electrolyte) are examined. When supporting electrolyte concentration is lower than 0.3M, peaks are broader that is due to presence of migration current (Fig. 2). At supporting concentration higher then 0.3M KCl peak areas become independent of supporting electrolyte concentration; This means almost all the migration current has been

compensate and 0.3M KCl is sufficient to use KNO_3 could also be used instead of KCl. Therefore, 0.3M KCl is chosen for further study (Fig. 2). Scan rate is also varied as shown in (Fig. 3). The optimum scan rate is $100mV.S^{-1}$ and 400 mV is optimum value for potential in measuring diffusion current for amperometric determination. Therefore $100mV.S^{-1}$ is used as scan rate throughout study. As plot between square root of scan rate and anodic diffusion current in such range of scan rate is straight line that complies Randles-Sevcik equation 1.1 [R.S. Nicholson and I. Shain, 2008] as shown in (Fig. 4).

$$Ip = 2.69\ 10^5\ n^{3/2}AD^{1/2}\nu^{1/2}c \quad ----------1.1$$

This equation also helps to investigate diffusion coefficient. Slope of the curve (Fig. 4) could be $2.69x10^5\ n^{3/2}AD^{1/2}C$. Diffusion coefficient is evaluated from the slope is about $7.6x10^{-4}$order since diffusion is also depending upon thickness of diffusion layer as given below therefore the value obtained for diffusion coefficient by slope of Randle-Sevick equation could be used to estimate thickness of diffusion layer using following equation (1.2) [David Harvey, 2000].where limiting current i_l is obtained at maximum concentration of analyte (i.e C_{bulk} at electrode of A surface area with diffusion co efficient D, thickness of diffusion layer δ is for n electrons involved in redox reaction.

$$i_l = \frac{nFAD}{\delta} C_{bulk} \quad --------1.2$$

About 13mm is found to be thickness of diffusion layer. This thickness is quiet feasible for usual voltammetry scans.

Risk of having any noticeable capacitive current is assessed by using Differential Pulse (DP) mode. Therefore, pulse of 100mV is overlapped with scan rate of $100mS^{-1}$. Each pulse is of 70ms duration. Currents are measured at two instants, 20ms just before pulse application and in last 20ms of pulse duration. No significant difference in current noticed before and during pulse. This shows that no or negligible capacitive current is present in system. Glassy Carbon (GC) electrode, Platinum (Pt) electrode, and Gold (Au) disc electrodes are employed as indicator electrodes. A good sensitivity is obtained for Glassy Carbon and thus selected for further analyses as shown in Fig. 5. Scan mode of linear scan and DP scan are compared. No significant difference is obvious. It also reflects absence of capacitive current. Area of electrode is varied and current response is according to Randles-Sevcik equation (i.e. good linearity). A circular disk of 0.07065 cm^2 cross sectional area is used throughout.

Reversibility of system is beyond consideration because no reduction peak is observed in cyclic voltammetry (Fig. 1). Amperometric conditions are also adjusted in consequence of CV. 400mv is chosen as applied potential (Fig. 3) and 3.0M KCl as supporting electrolyte at Glassy Carbon (GC) as indicator electrode against Saturated Calomel Electrode (SCE) as reference and Platinum (Pt) as auxiliary electrodes.

A good quality calibration curve has been established by running Vitamin C standards. Blank solution are run in replicates and detection limit is estimated using equation (detection limit = $Y_b \pm 3S_b$). Therefore, Detection limit is found $1x10^{-3}$mM. Sensitivity of calibration curve is $13.5mA.M^{-1}$ with linear range of 0.05 to 0.5mM ascorbic acid.

As far as medicine or drugs are analyzed it shows shift is in signals for oral medicines (Fig 6). That is due to interference in redox reactions at working electrode. This is showing poorer selectivity.

Usually drugs are having exipients that provides heavy matrix in analysis. That could interfere the redox system of vitamin-C. Negligible interference affects are obvious in injectables and capsules. Shifting in voltammogram peak is more obvious in runs of tablets and syrups. In order to detect chemical agents that are affecting voltammetry, a study is designed is for investigation of possible interferences. In syrups major components are sugar, glucose, alcohol. Beside with these, some compounds that are usually added as flavoring agent, coloring agent, stabilizer etc could interfere in cyclic voltammetry. Sugar is added to cyclic voltammetry run of vitamin-C under selected conditions. Effects of shifting in peaks are observed. Different coloring agents and flavors are also assessed for possible interference. Fortunately, such interference is not in quantitation of ascorbic acid but it just shifting the potential (i.e. qualitative effects).

Therefore it is easier and more feasible to employ standard addition method or spiking standards than removal of interference. Such removal usually demands special digestions. That may be tedious chemical or physical treatments. This could also cause loss in vitamin-C content in such treatments. Therefore, spiked samples are run that shows good agreement with qualitative and quantitative voltammetry (Fig. 7). Shifting in peaks may be due to modification in supporting electrolyte in presence of excipients. Comparisons of Fig. 6 and 7 establish that it is good to employ standard addition methods in order to avoid interference effect. Recoveries for Vitamin-C for such medicine are estimated to be around 100±5% for spiked samples (Fig. 7). Although there is no need of spiking in case of injectables and oral drops.

Table 1: Conclusion of results obtined in different products.

S. No.	Sample Type	Method	Ascorbic Acid potency as per label clam (mg)	Amount (mg)	Relative Error
1	Core tablet	[1]Std. Add	500	512	2.40%
2	Capsule	Std. Add	500	506	1.20%
3	Syrup	Std. Add	100	113	13%
4	Oral Drops	[2]Cali. Mtd	100	108	8%
5	Injectables	Cali. Mtd	500	503	0.6%
6	Powdered Drinks	Std. Add	100	112	12%

[1] Standard Addition Method
[2] Calibration Curve Method

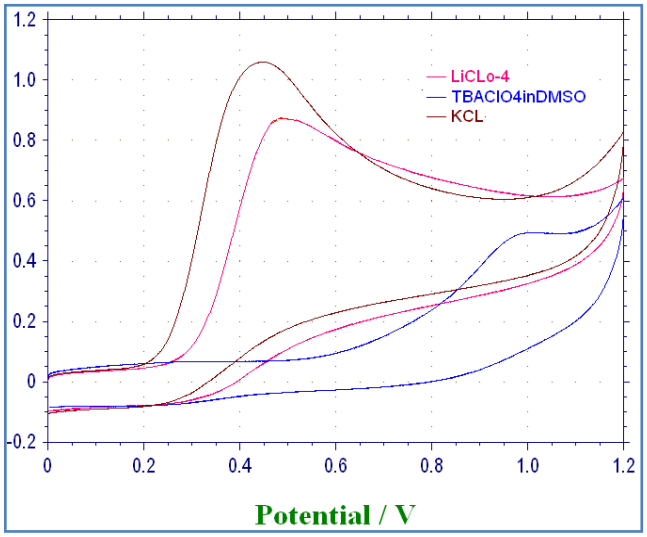

Fig 1. Cyclic Voltammogram of ascorbic Acid in presence of 0.3M of Different Supporting Electrolytes at scan rate of 100mV.S^{-1}

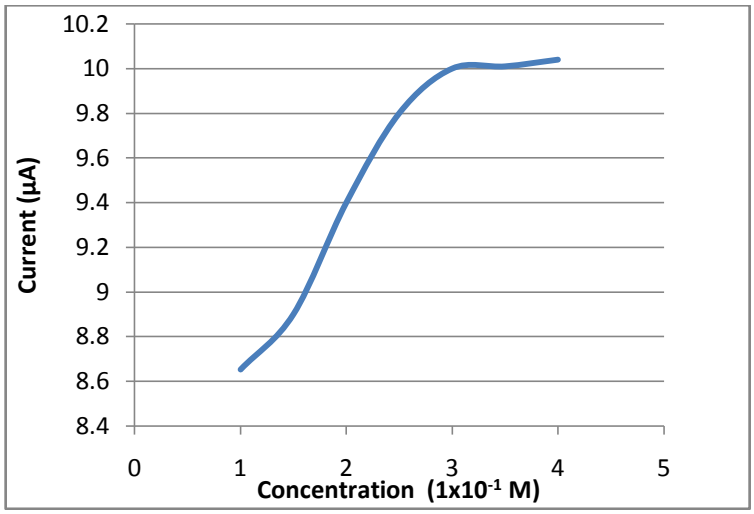

Fig. 2 Diffusion current of Ascorbic Acid with Different Concentration of KCl as Supporting Electrolyte.

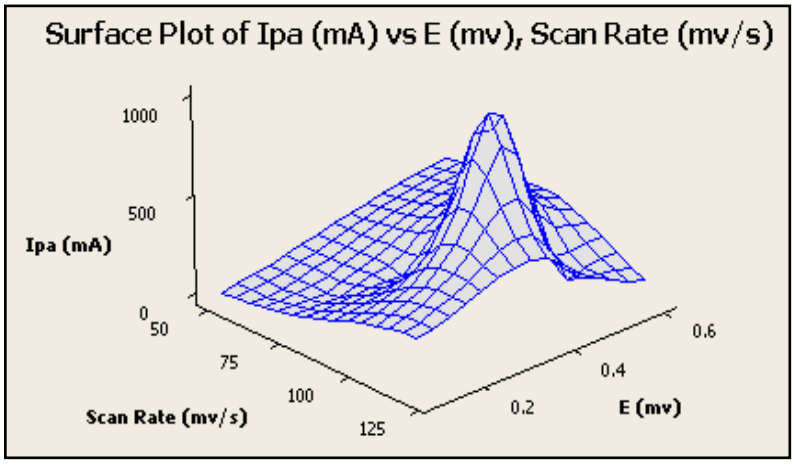

Fig. 3 ; Three dimentional curve showing optimum conditions for doing cyclic voltametry of vitamin C.

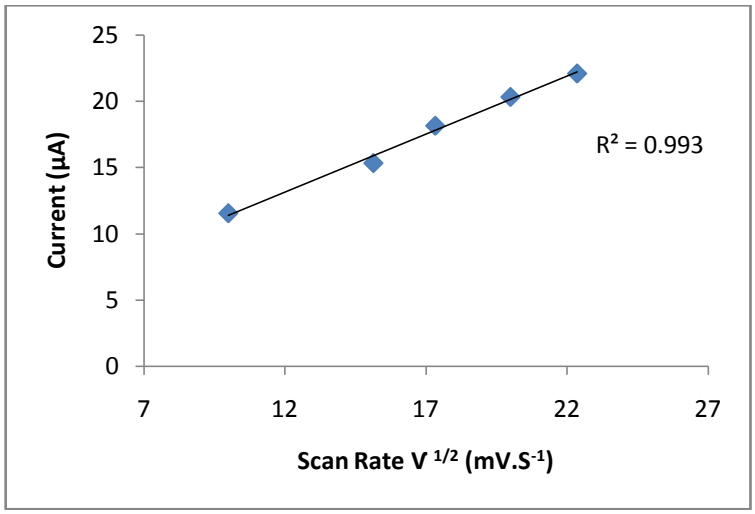

Fig. 4 Squre Root of Scan Rate Verses Current

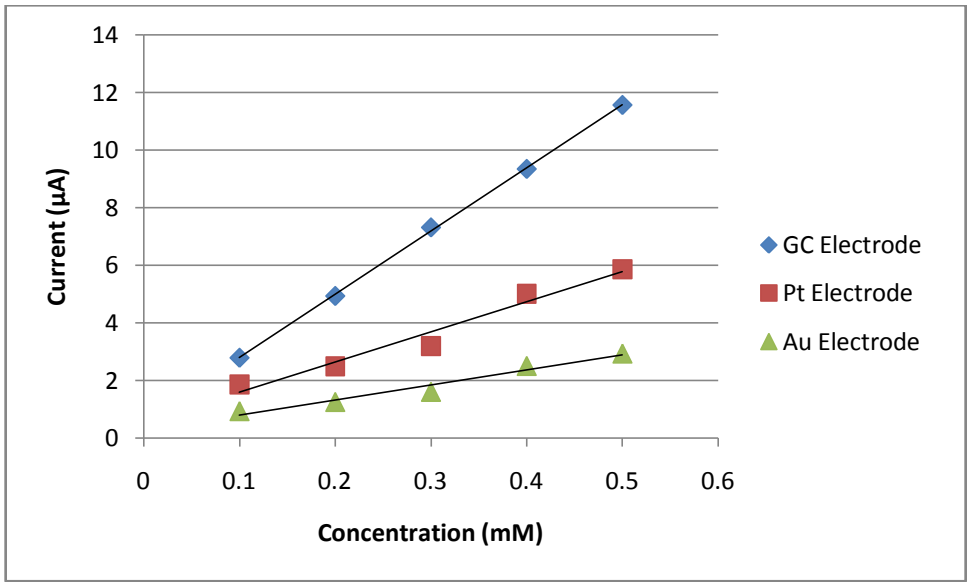

Fig. 5 Concentration of Ascorbic Acid at 100mV.S^{-1} for different indicator electrodes

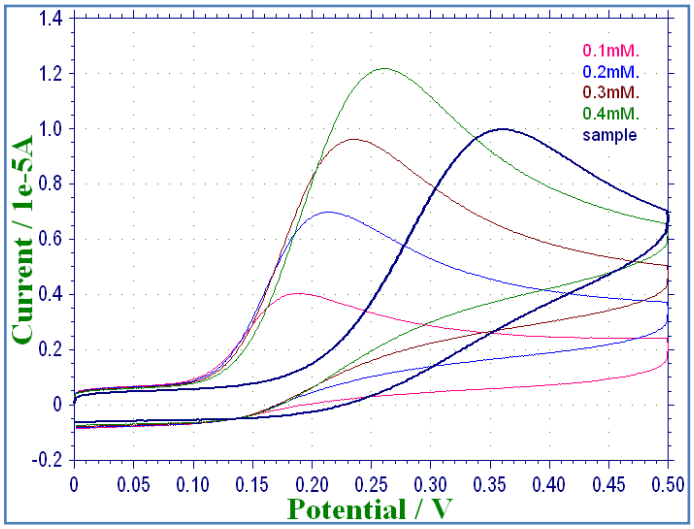

Fig. 6 Cyclic Voltammogram of Standards and one Sample.

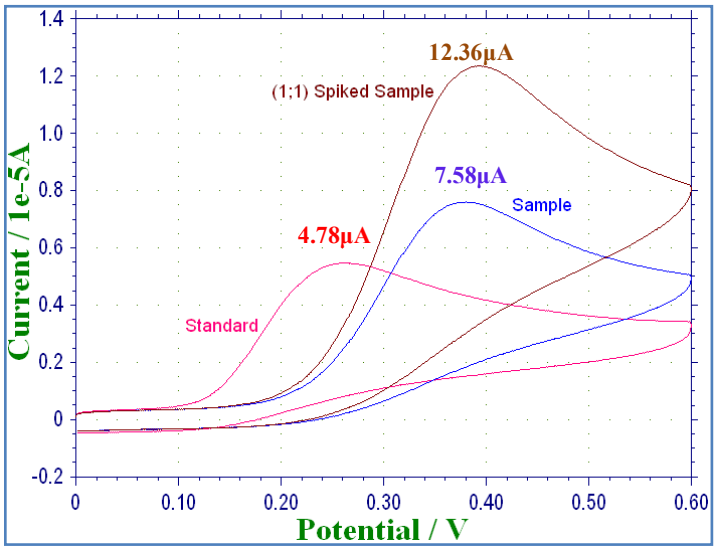

Fig. 7: Cyclic voltammogram for standard vitamin C vitamin –C in sample Spiked or (1:1) Mixture of standard and sample.

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