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Improvement of the electrochemical determination of antioxidant using cationic micellar environment

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ABSTRACT. Ascorbic acid (AA) is an important compound of the human diet, in the metabolism acting mainly as exogenous antioxidant. A method for electrochemical detection of Ascorbic acid (AA) with a GCE was developed using a cationic surfactant cetylpyridinium chloride (CPC). The presence of CPC influences the electrochemical behaviour of AA, reduces the applied potential to measure AA oxidation followed by an increase in the peak current. In the optimized conditions the AA determination was possible in the linear range of 5.0 x 10^{-7} mol L^{-1} - 4.3 x 10^{-4} mol L^{-1} with a detection limit (S/N = 3) of 2.0 x 10^{-7} mol L^{-1}. The applicability of the method is in the sensitivity and selectivity of the GCE for the AA detection as well as the simplicity of the demonstrated method using an electrode without modifying and without pre-treatment of samples. Recovery tests presented values of 98 - 103% suggesting the method has no significant interference and making possible to be successfully used for AA determination in complex samples.

Key words: ascorbic acid, micellar system, cationic surfactant.

RESUMO. Determinação eletroquímica de antioxidante em meio micelar catiônico.O ácido ascórbico sendo um componente essencial na dieta humana, apresenta grande interesse biomédico por executar funções essenciais no metabolismo de mamíferos, atuando principalmente como antioxidante exógeno. No presente trabalho é apresentado um procedimento simples e rápido para determinação eletroquímica de ácido ascórbico (AA) em urina, fármaco e suco de laranja "in natura", utilizando eletrodo de **carbono vítreo** (GCE) em meio micelar contendo cloreto de cetilpiridínio (CPC). A presença de CPC afeta o comportamento eletroquímico do AA, reduz o potencial de pico eletrodo, bem como aumenta a intensidade da corrente de pico anódica. Em condições otimizadas, a determinação de AA foi possível na faixa linear de 5,0 x 10^{-7} mol L^{-1} – 4,3 x 10^{-4} mol L^{-1} com um limite de detecção de 2,0 x 10^{-7} mol L^{-1}, considerando uma relação sinal/ruído = 3. A aplicabilidade do método foi demonstrada por estudos de recuperação para a detecção de AA nas amostras sem pré-tratamento. Os resultados mostraram bons percentuais de recuperação (98 - 103%) sugerindo que o método proposto não sofre influência significativa da matriz mostrando grande potencial de aplicação.

Palavras-chave: ácido ascórbico, sistema micelar, surfactante catiônico.

Introduction

Ascorbic acid (AA) (γ-lactone) is a water-soluble compound cited as antiscorbutic and antioxidant playing a key role in the immune response. This dietary component is well-known as an effective chain-breaking antioxidant employed as additive in emulsions to prevent lipid oxidation in pharmaceutical preparations and food. Thus, the oxidation of this compound to L-dehydroascorbic acid has received a great attention because of the important role that the redox chemistry of AA plays in human nutrition. The acid character and reduction action are attributed to its enodiol group (-COH=COH-) in the structure (LIU; ANZAI, 2004).

Considering the therapeutic purposes, nutritional assessment and important industrial application of AA, its determination in a selective and simple way has been a subject of constant interest. Among the analytical procedures to quantify AA, electrochemical approaches seem to have the greatest versatility (ARYA et al., 2000). However using bare electrode for instance is difficult because its fouling by oxidation and formed interfering products (ZEN et al., 2003). Strategies to eliminate interferences include covalent attachment of moieties with antifouling properties or ultrasonication (BANKS; COMPTON, 2003). Electrochemical treatment of working electrodes as glassy carbon electrode by potential cycling or

prenodization has also been used to minimize adsorption effects in electrochemical determination of organic compounds (DOWNARD; RODDICK, 1994).

Recently has been shown the anionic surfactant sodium dodecyl sulfate (SDS) is effective in suppressing or eliminating adsorption interferences from proteins and phospholipids in the voltammetric determination of several bioorganic compounds. The anti-fouling effect of the SDS is attributed to its property of formation soluble aggregates with surface-active compounds (HOYER; JENSEN, 2005). It is known that surfactant behavior in solution involving aggregation or adsorption and organization on the electrode surface and it influences the electrode processes and the rate of electron transfer (VITTAL et al., 2006). In addition it has been reported that the antioxidant activity of AA was enhanced in micelles systems in some study of the anodic oxidation of AA and its lipophilic derivates in the presence of micelles (JAISWAL et al., 2001). The effect of micelles on chemical reaction has been drawing interest of many researchers, since chemical reactivity, equilibrium and stereochemistry of the reactants are significantly affected by micelles. Micellar catalysts of reactions are important due to their industrial applications pertinence to biological processes and analytical chemistry and also because of the parallel behavior of biological macro-molecules and enzymes. Micellar catalysis of reaction in aqueous solution is generally explained in terms of distribution of reactants between water and micelles with reactions occurring in both media (PENG; GAO, 2006).

Therefore studies in this direction can contribute to a better understanding of the role of surfactant in the improvement of analytical determination of biologically relevant compounds as AA.

This work describes a fast and simple method to determination of AA using a glassy carbon electrode without modification in the presence of cationic CPC micellar system. The surfactant works as masking agent and influences positively the electrochemical oxidation of AA. As a result is observed an improvement in the selectivity and sensitivity of the proposed method.

Material and methods

Reagents and Solution

Ascorbic acid (AA) and cetylpyridinium chloride (CPC) were used without further purification and were purchased from Sigma (St. Louis, USA). All aqueous solutions were prepared using ultrapure water (ρ > 18 MΩ cm^{-1}) from a Milli-Q System (Millipore, System). All other used chemicals were analytical grade reagent.

Apparatus and Equipments

The electrochemical measurements were carried out in phosphate buffer solution (PBS) 0.1 mol L^{-1} using a conventional cell based on satured calomel electrode (SCE) as reference, platinum wire as auxiliary and glassy carbon electrode (GCE) as working electrode. The used CGE (A = 0.071 cm^2) was polished with Al_2O_3 (0.5 μm), followed by rinsing with deionized water and then sonicated using alcohol and deionized water for 2 min.

A potentiostat from Eco Chemie Autolab® PGSTAT30 connected to a PC (software GPES 4.8) from Eco Chemie (The Netherlands) was employed in the measurements. Electrochemical experiments were performed at room temperature.

Results and discussion

Shows the effect of the CPC on the electrochemical oxidation of AA in PBS. Voltammetric curves of AA recorded in the absence of CPC (Figure 1b) showed an irreversible cyclic voltammogram with the AA oxidation peak around +0.23 V (*vs* SCE). Details of AA's electrochemical mechanism in bare CGE were reported in previous work (HU; KUWANA, 1986) and include an oxidative mechanism of multi steps (Figure 1).

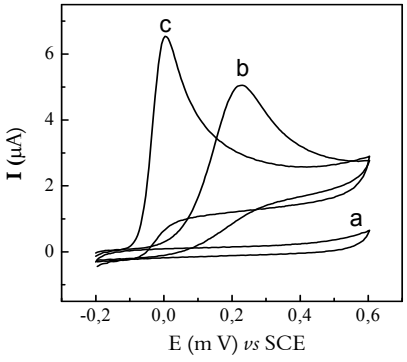

Figure 1. Cyclic voltammograms of 200 μmol L^{-1} AA recorded at a GCE in 0.1 mol L^{-1} PBS (pH 7.0); scan rate: 100 mV s^{-1}: (a) blank; (b) absence and (c) presence of 1.0 mmol L^{-1} CPC solution.

The presence of CPC in solution promoted an increase in the electron transfer for the oxidation of AA as observed by the shift of the anodic peak potential toward less positive values followed by an increase in the peak current. Compared with bare GCE, the system in the presence CPC exhibits a 220 mV shifts of the oxidation potential of AA in the cathodic direction and a market enhancement of the current response.

The observed changes in the redox parameters of AA can be attributed to a surface effect. It is well established that surfactants can be absorbed on solid surfaces to form surfactants films (ROY et al., 2003; SZYMULA; MICHALEK, 2003). This behavior can be explained considering that AA in the studied media (pH 7.0) is monoprotonated and in micellar medium, before the CMC, this dissociated form is stabilized by an electrostatic interaction with CPC. A possible adsorption of the surfactant onto electrode surface may result in the formation of a positively charged hydrophilic film with the polar head group towards to the bulk of the solution, which interact with anionic AA. As a result a great effect is observed on the electron transfer rate.

Previous tests were performed to verify if occurs the formation of CPC film on the electrode and if this is the responsible by the observed electrochemical behavior. The film was prepared in a concentrated CPC solution before measuring. After immersing the electrode in a 0.1 mol L^{-1} CPC solution during some minutes and after washed with water, the cyclic voltammogram recorded in presence of AA without CPC in the medium was similar to those obtained in the CPC presence, indicating the CPC film adsorbed on the electrode surface.

This effect on the behavior of AA was found to be dependent on the CPC concentration as shown in Figure 2. The oxidation potential of AA decreases with the increasing of the surfactant concentration. In the vicinity of CMC (critical micellar concentration) reaches a plateau corresponding to 0.020 V, whereas the peak current increases significantly up to the CMC and after this value it remains constant.

In the vicinity of CMC, ascorbate acid anion and the cationic surfactant coexist in solution in dynamic equilibrium, which starts the attachment of the anion radical to the micelle. At the CMC, in the present study, estimated by cyclic voltammetry occur the micellization process. The micellar assembly can be formed by the positively charged polar head group of surfactant locate in the interfacial region of the micelle and in its external core are the ascorbic acid molecules (ascorbate anions) bind by electrostatic interaction. After the CMC is observed a plateau in the plot of E_{pa} and I_{pa} against the surfactant concentration demonstrating a saturation of the adsorbed surfactant onto electrode surface. The excess of surfactant form micelles in the bulk solution and it no longer affect the electron-transfer process. Thus, it is possible to verify that CPC concentration around 1.0 mol L^{-1} was enough to reach the CMC. Hence, this value was chosen for further experiments.

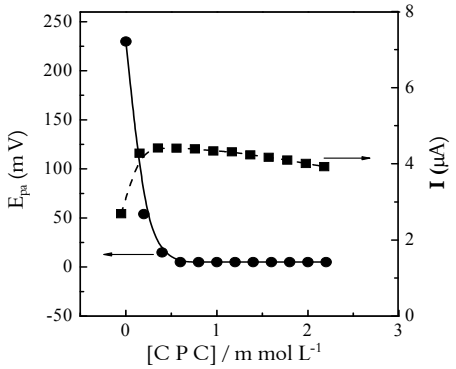

Figure 2. Variation of anodic peak potential (E_{pa}) and anodic peak current (I_{pa}) of AA at a GCE with the surfactant concentration. Experimental conditions as in Figure 1.

Optimized analytical parameters

In the electrochemical determination of organic molecules, the applied potential has a great influence over to the analytical response (SRIPRIYA et al., 2006). The dependence of potential on the electrode current is shown in Figure 3. It can be seen that the applied potential 0 V vs SCE is adequate to reach the maximum response for AA in CPC micellar medium. The presence of surfactant in the medium allows that redox direct reaction of AA at bare conventional electrodes as GC, occur in low potential. This eliminates the pronounced fouling effect associated with the oxidation of interference commonly present in samples, since this problem result in rather poor reproducibility to determine the vitamin. Thus, this potential was fixed for further analytical determination.

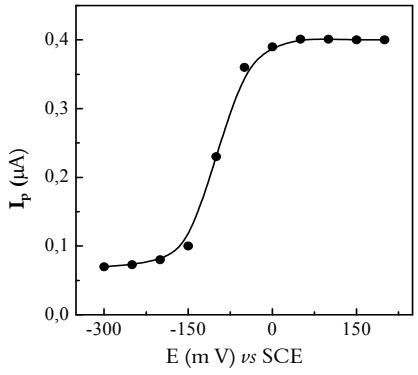

Figure 3. Influence of applied potential on the GC electrode response for AA in the presence of 1.0 mmol L^{-1} of CPC solution. Measurements carried out in 0.1 mol L^{-1} PBS (pH 7.0).

The amperometric determination of AA based on its electrochemical oxidation can be done at GC

electrode operating at potential above + 500 mV *vs* ECS. This overpotential could be lowered substantially by the presence of surfactant in the medium as can be seen in Figure 4 that presents the changes in the steady-state oxidation current at 0 V *vs* SCE on the stepwise addition of increment of 50 μmol L^{-1} AA into PBS. The electrode response in the presence of surfactant is attained with much better sensitivity and more extensive linear response range in comparison those without surfactant in medium. The effect of surfactant on the performance of the GC electrode is attributed to the interference suppressor effect that allows the direct determination of AA (CONNORS et al., 1985; KAMAU et al., 1987) in this low potential with great sensitivity. This result is significant from the viewpoint of its practical application.

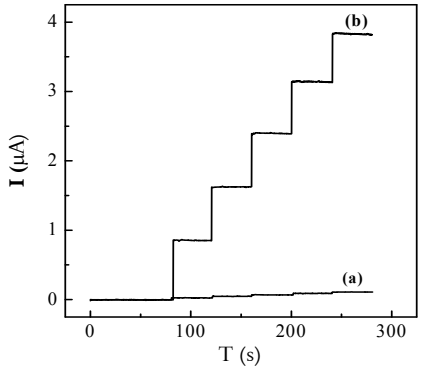

Figure 4. Amperometric response obtained with GCE for AA in the 0.1 mol L^{-1} PBS solution (pH 7.0) (a) in the absence and (b) presence of 1.0 mmol L^{-1} CPC solution. Applied potential of 0mV *vs* SCE, increments of 50 μmol L^{-1} AA and rotating speed: 200 rpm.

The GC electrode response for AA in the presence of 1.0 mol L^{-1} CPC showed a linear range from 5.0 x 10^{-7} mol L^{-1} up to 4.3 x 10^{-4} mol L^{-1}, expressed by the linear regression equation $I_{(\mu A)}$ = 0.05 (±0.01) + 0.017 (±0.001) [AA] μmol L^{-1}, with a correlation coefficient of r = 0.9996 (n = 9). The detection limit of 2.0 x 10^{-7} mol L^{-1} (S/N = 3). The present method showed sensitivity values lower than those obtained in recent reports for AA determination based on chemically modified electrode (CME). In these studies, the values obtained were linear range from 5 x 10^{-6} to 1 x 10^{-4} mol L^{-1} and DL = 2.45 x 10^{-6} mol L^{-1} (O'CONNELL et al., 2001), linear range from (2 – 4) x 10^{-6} mol L^{-1} and DL = 6 x 10^{-7} mol L^{-1} (FEI et al., 2004), linear range up to 6.2 x 10^{-3} mol L^{-1} and DL = 3.1 x 10^{-7} mol L^{-1} (MOTLAGH; NOROOZIFAR, 2003).

The repeatability in the measurements was evaluated through a series of 10 repetitive experiments carried out with 40 and 200 μmol L^{-1} ascorbic acid solutions. The relative standard deviations (R.S.D.) were 1.5 and 0.5%, respectively. These results suggest a good precision of the proposed method.

The response time considering the time to reach 100% of the signal was around 2 s. The response time was very better than the absence of CPC (around 20 s) at the same conditions of potential and concentration.

AA determination in samples

The fruit juice samples were diluted 100 times with PBS (pH 7.0) before measurements. Commercials pharmaceuticals were tested of fixed contents (300 mg $tablet^{-1}$). A known amount of one tablet equivalent to 15 mg of AA was diluted in 10 mL electrolyte solution. The urine samples were tested without pretreatment. The AA content of the samples solution was detected using a conventional technique of standard addition method by extrapolating the linear curve. The accuracy of the results was verified by the recovery tests using samples in triplicate. In the samples were added certain amounts of AA in about the same concentration as found in the samples themselves. As shown for samples of orange juice and urine, recovery values were close to 100%. Samples of drug, despite simpler composition, showed major variations which can be attributed to an interference of matrix or other factor such as, errors in the experimental procedure as ineffective cleaning of electrode before measuring.

Considering the variety of interferents compounds coexisting in the tested samples the practical usefulness of the proposed method was demonstrated, which indicating that the proposed method can be applied very well in the analysis of the samples (Table 1).

Table 1. Determination of AA by proposed method.

Sample	[AA] added	[AA] found*	Recovery (%)
Human urine 1	80.0 μmol L^{-1}	82.0 ± 2.0 μmol L^{-1}	103.0
Human urine 2	80.0 μmol L^{-1}	79.2 ± 1.0 μmol L^{-1}	99.0
Human urine 3	80.0 μmol L^{-1}	80.4 ± 1.0 μmol L^{-1}	102.0
Vitamin C tablet 1**	6.0 mg L^{-1}	6.1 ± 1.3 mg L^{-1}	100.2
Vitamin C tablet 2**	6.0 mg L^{-1}	5.9 ± 1.0 mg L^{-1}	98.3
Orange juice 1***	6.0 mg L^{-1}	6.0 ± 0.2 mg L^{-1}	100.3
Orange juice 2***	6.0 mg L^{-1}	6.1 ± 0.2 mg L^{-1}	101.0

*Standard deviation for three replicates; **label value = 300 mg AA; ***mg AA calculated by the method proposed = 485.2 ± 10 mg L^{-1}.

Conclusion

The positively charged CPC adsorbed onto the electrode surface is responsible to control the electrode reactions of AA, shifting its oxidation

potential toward less positive values. Thus, the oxidation of AA is facilitated at the positively charged surfactant CPC in solutions by electrostatic interaction between AA and CPC.

The electrode response to determine AA, in the presence of the CPC surfactant was very sensitive and good values in the recovery tests were found, contribute to the applicability of the proposed method for AA determination in complex samples.

Considering the analytical features of the method it is possible to conclude that it presents advantageous characteristics when compared to other similar methods mainly due to the simplicity, since used a GC electrode without modification.

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