Short Communication

Electrocatalytic Determination of Vitamin C Using Calixarene Modified Carbon Paste Electrodes

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Abstract
Effect of in situ complexation of some ions with variable valencies, like Co(II), Ni(II), Mn(II), Cu(II) and Pb(II) on the the electrooxidation of Vitamin C (L-ascorbic acid) was studied by cyclic voltammetry using carbon paste electrodes modified with *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[6]arene in perchloric acid, acetic acid and ammonium acetate media. Pb(II) was found to bind strongly with *p*-tert-butylcalix[4]arene in acetate medium, resulting in its being retained at the electrode surface and catalyzing the oxidation of ascorbic acid. The overpotential was reduced by about 200 mV with an increase in the peak currents. Linearity was observed over the range of 0.07 – 400 ppm with a detection limit of 30 ppb by differential pulse voltammetry. Interferences of some common substances like sugars and amino acids were studied and the modified electrode was used for the determination of vitamin C in commercial samples.

Keywords: Carbon paste electrode, Calixarene, Ascorbic acid, Voltammetry

Vitamin C (L-ascorbic acid), a water-soluble vitamin that is widely required for metabolism and consumed on a large scale is electroactive and has been studied extensively [1 – 4]. Several chemically modified electrodes (CMEs) have been fabricated, and ascorbic acid has been used as a model compound to study the effect of modification, which in-turn have led to a number of electrochemical sensors. CMEs based on enzymes [5 – 7], polymerization [8 – 13], dyes [14 – 17], self assembled monolayers [18 – 21], redox [22 – 23] and macrocyclic complexes of transition metal ions are reported.

Most of the macrocyclic complexes used as modifiers are phthalocyanines [24 – 26], porphyrins [28 – 29] or cyclam type of molecules [21, 30, 31]. However, to the best of our knowledge, there is no report on the utility of calixarene-metal ion complexes in electrocatalysis, though they have been used in a number of ion selective electrodes (ISEs) [32 – 35] and CMEs [36 – 40]. Therefore, we considered it interesting to study if the calixarene complexes exhibit similar electrocatalytic activity as the other macrocyclic compounds mentioned above. The calixarenes chosen were, *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[6]arene for their cavity radii are closer to the ionic radii of the commonly used transition metal ions which exhibit multiple valencies, viz. Co(II), Ni(II), Mn(II), Cu(II). Pb(II) was also included in our study as it is known to form stronger complexes [32, 36] with calixarenes than the above mentioned ions, and in fact were found to be the only complexes which exhibited electrocatalytic behavior.

A reaction at an electrode is said to be electrocatalytic if the oxidation/reduction potential is decreased considerably and if there is a simultaneous increase in peak currents. The oxidation of ascorbic acid (AA) was studied in 0.01 M perchloric acid (pH 2.0), 0.04 M acetic acid (pH 3) and ammonium acetate media (pH 5.1) containing Cu(II), Co(II), Ni(II), Mn(II) and Pb(II) ions using plain carbon paste electrodes (PCPEs) and modified carbon paste electrodes (MCPEs). Electrocatalytic activity was observed only for the MCPEs in ammonium acetate media containing Pb(II) ions, which is consistent with previous observation that the uptake of lead ions is favored in ammonium acetate medium [41]. No electrocatalytic effect was observed in the presence of other cations suggesting that their complexation with calixarenes was not favorable in the studied media. As for Cu(II), its presence caused rapid auto-oxidation of ascorbic acid. Figure 1 shows the cyclic voltammograms (CVs) for the oxidation of 4×10^{-4} M ascorbic acid in 0.01 M ammonium acetate, with and without 5 mM $Pb(NO)_3$. It is clearly seen that, in the absence of complexed lead ions at the electrode surface, the oxidation at PCPE or MCPEs show long drawn out peaks at higher positive potentials; whereas, the peaks obtained by *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[6]arene modified electrodes, MCPE-I and MCPE-II respectively are sharper, higher and at less positive potentials in presence of lead ions. The oxidation peak potentials at MCPE-I and MCPE-II are about 250 mV and 200 mV more negative than at PCPE, which is an indication of electrocatalytic behavior. Though the mechanism of electrocatalysis is not clearly understood at this stage, and since there is no oxidation or reduction of Pb(II) within the applied potential range; this can be considered as an example of supramolecular metallocatalysis, wherein the complexed metal ions provide a means of substrate activation resulting in easy oxidation [42]. The greater catalytic activity of MCPE-I can be explained by taking into

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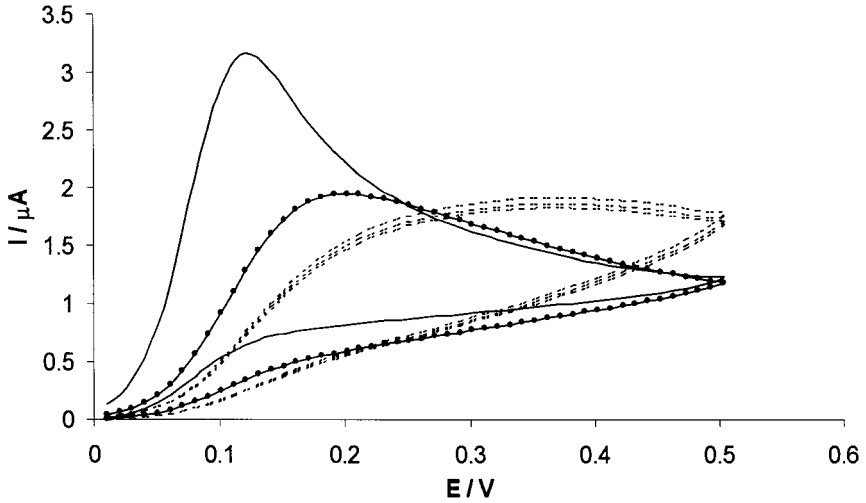

Fig. 1. Cyclic voltammograms at 50 mVs^{-1} for 4×10^{-4} M ascorbic acid in 10 mM CH_3COONH_4. (------) lower curve, with PCPE and without Pb(II) ions; (------) middle curve, with MCPE-I and without Pb(II) ions; (------) upper curve, with PCPE and with 5 mM Pb(II) ions; (●–●–●) with MCPE-II and with 5 mM Pb(II) ions; (——) with MCPE-I and with 5 mM Pb(II) ions.

consideration, the relative sizes of the calixarene cavities and diameter of lead ion. It is known that divalent lead (2.36 Å) forms more stable complex with *p*-tert-butylcalix[6]arene (2.1–2.8 Å) than with *p*-tert-butylcalix[4]arene (0.68–0.92 Å), due to the close match of its cavity size with that of the lead ion in dimethyl formamide [32], though no such complexation studies in aqueous media are reported due to their insolubility in water. So, based on the relative sizes and cation–π interaction [43], it is expected that the lead ion would be held deeper into the cavity of *p*-tert-butylcalix[6]arene and rather at the rim of the *p*-tert-butylcalix[4]arene cavity, enabling it to interact more freely with AA on the solution side, thus acting as a bridge for electron transfer [42].

As is known the oxidation of ascorbic acid is pH dependent [44], the effect of pH on the electrocatalytic behavior was studied by varying the pH of the supporting electrolyte 10 mM $CH_3COONH_4 + 5$ mM $Pb(NO_3)_2$ with addition of acetic acid or sodium hydroxide. Figure 2 shows the CVs obtained for 4×10^{-4} M AA at different pH using MCPE-I. It is seen that, with an increase in pH, there is a shift in the peak potential towards less positive values, which is also true in case of any bare electrode, for example, gold [44]. However, in these cases the shape and height of the peaks are independent of pH. With the present electrode (MCPE-I), the lower peak heights at pH 3.1 and 4.1 can be attributed to absence or reduced complexation of Pb(II) at the surface. The peak at pH 6.1, though at less positive potential is smaller, because of precipitation of Pb(II) which was observed to begin at a pH of about 5.9. So, as the lead ions are prevented from forming a complex with the calixarene at the electrode surface (either by increasing or decreasing the pH), there is a decrease in the catalytic activity.

From the above studies, it is clear that 10 mM $CH_3COONH_4 + 5$ mM $Pb(NO_3)_2$ is the most suitable medium (which has a pH of 5.1) and MCPE-I, the best electrode for electrocatalytic oxidation of ascorbic acid. Varying the

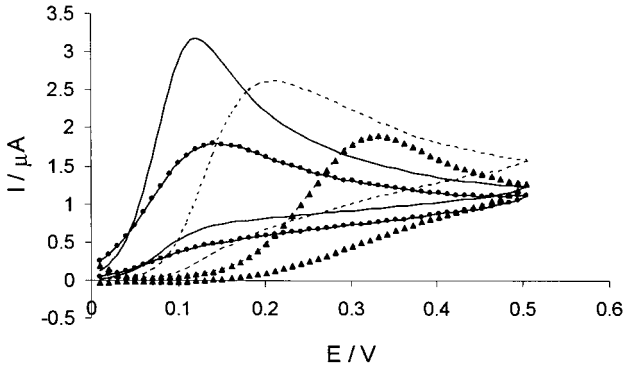

Fig. 2. Cyclic voltammograms for 4×10^{-4} M ascorbic acid in 10 mM $CH_3COONH_4 + 5$ mM $Pb(NO_3)_2$ at 50 mVs^{-1}, using MCPE-I at pH 3.1 (▲–▲–▲), 4.1 (------) , 5.1 (—) and 6.1 (●–●–●).

concentration of Pb(II) between 3–8 mM did not cause any change in the response, but at concentrations lower than 3 mM, the electrode required some time to equilibrate (complexation at all the available calixarene sites) before showing catalytic response. So, further studies were carried out with MCPE-I in 10 mM $CH_3COONH_4 + 5$ mM $Pb(NO_3)_2$.

The effect of scan rate was studied by cyclic voltammetry and the anodic peak current was found to increase linearly with the square root of scan rate in the range 10–500 mVs^{-1} which indicates a diffusion controlled reaction. For the purpose of quantification, differential pulse voltammetry (DPV) was used. A linear relation was found between the concentration and peak current over the range of 0.07–300 ppm (slope = 36.2 nA/ppm, intercept = 331 nA, correlation coefficient = 0.9931) with a detection limit of 0.03 ppm (RSD = 4.9 % for $n = 6$). Whereas, with the PCPE, linearity was observed over the range 0.6–300 ppm (slope = 15.7 nA/ppm, intercept = 220 nA, correlation co-

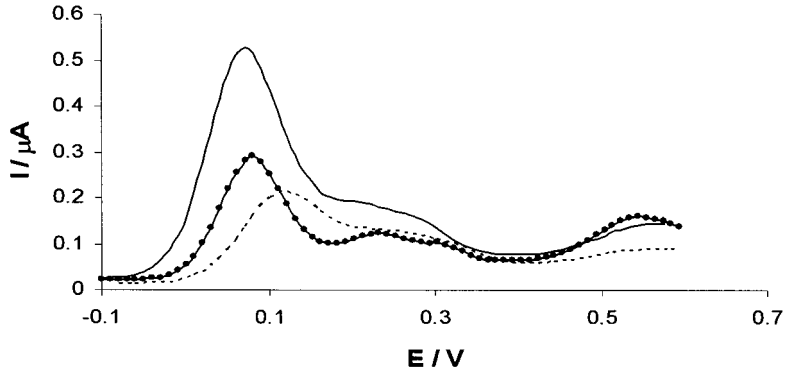

Fig. 3. Differential pulse voltammograms of multi-fruit juice sample, diluted 50 times with 10 mM CH_3COONH_4 + 5 mM $Pb(NO_3)_2$. PCPE (------), MCPE-I (●–●–●) and MCPE-I (—) after addition of 0.1 mL of 880 ppm AA. Scan rate = 10 mVs^{-1} and pulse amplitude = 50 mV.

efficient = 0.9957). However, linearity was observed up to 400 ppm (correlation coefficient = 0.9994) when the area under the peak was considered instead of the peak height, with MCPE-I. At higher concentrations, there was considerable peak broadening, hampering the linearity. Interference effects were studied by adding large excesses of sugars, amino acids and metal ions to a solution containing 4 ppm ascorbic acid. It was found that, hundred times excess of glucose, sucrose, mannitol, sorbitol, alanine and glycine did neither effect the peak height nor its position, when present individually or as mixtures. The common metal ions like Co(II), Ni(II), Mn(II), Zn(II) also did not interfere when present in 100 times excess concentrations. But hundred times excess of cysteine caused a lowering of peak height by about 20% and also the peak potential was shifted to more positive potentials by about 30 mV. However, in real samples, like pharmaceutical preparations or fruit juices, these interferences are not present at such high concentrations. Table 1 shows the results of analyses performed on some commercial samples.

It can be seen that the present electrode and method display sufficient accuracy and precision for real sample analyses. Figure 3 shows representative DPVs of the multi-fruit juice sample which were obtained for the assay of AA by standard addition method. The same technique was used for all other samples. A DPV obtained with PCPE is also shown for comparison.

The Pb(II) ions incorporated into the cavity of calixarenes via in situ complexation can catalyze the electrochemical oxidation of ascorbic acid. Though the exact mechanism is not known at present, this electrocatalytic behavior causes an increase in the peak currents, thereby increasing the sensitivity of the technique. Electrocatalysis also shifts the oxidation peak potential to less positive values which can help in avoiding interfering signals as is demonstrated in Figure 3, or provide means for simultaneous analyses of different analytes like dopamine, uric acid, etc. which occur together with AA in biological samples. Further studies with such different analytes may lead to a better understanding of this electrocatalytic activity. As, the same electrode surface can be used for 12–15 times (RSD = 3.5 % for $n = 12$) with in situ electrolytic cleaning cycles in between, it may also be used in automated flow systems. After using the same surface for about 15 times repeatedly, a gradual drift is observed (broadening of the peak and decrease in peak heights, probably due to incomplete removal of reaction products at the surface). The relative standard deviation for manually renewed surfaces was 2.3 % ($n = 5$) and 3.1% ($n = 5$) for separately prepared carbon pastes. In contrast to the modified electrodes reported earlier [8–31], which involved tedious synthetic procedures of macrocyclic complexes or self-assembly or electropolymerization, the preparation of the proposed electrode is quite easy; being just a physical mixing of the commercially available ingredients. Thus, the proposed electrode and method provides a means of simple and elegant way of assaying vitamin C in commercial samples and the phenomenon of electrocatalysis by in situ complexation may be further extended for other organic compounds.

Table 1. Assay of Vitamin C in commercial samples ($n = 3$)

Sample	Quoted content	Observed content
Algidol (powder)	500 mg/sachet	497 ± 3
Aspirina-C	240 mg/tablet	242 ± 3
Calcinatal	50 mg/tablet	52.3 ± 1.5
Femivit	60 mg/capsule	61.4 ± 2.2
Fruit juice (pineapple)	30 mg/100 mL	31.2 ± 1.8
Fruit juice (multi-fruit)	30 mg/100 mL	32.8 ± 2.7

Experimental

Apparatus

An Autolab PSTAT 10 potentiostat with GPES software was used for recording the cyclic and differential pulse voltammograms. Carbon paste electrodes were used as working electrodes, with a gold electrode as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

Chemicals and Solutions

All chemicals were of analytical grade. Ascorbic acid, alanine, glycine, cysteine, glucose, sucrose, mannitol, sorbitol, graphite powder (1 – 2 μm) and mineral oil were from Aldrich, while the metal salts of lead, cobalt, copper, manganese, zinc and nickel, and perchloric acid, acetic acid, ammonium acetate, *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[6]arene were from Merck. Ultra pure water of Millipore grade (18 MΩ) was used for all experiments.

Electrode Preparation

Calixarene coated graphite powders were prepared as previously reported [45] by dispersing a weighed amount of calixarene in acetone, adding the required amount of graphite powder and stirring the slurry thus formed until all the acetone had evaporated. The modified pastes contained 5% (w/w) of calixarene w.r.t. graphite powder. The same surface of the carbon paste electrodes could be used for 12 – 15 analyses (the surface being cleaned in between by applying four consecutive potential cycles between 0.0 and – 0.3 V at 50 mVs^{-1}). For further use of the carbon paste electrode, the surface had to be regenerated by pressing out about 1 mm of the paste.

Cyclic voltammograms were recorded for ascorbic acid in 0.01 M perchloric acid, 0.04 M acetic acid and 0.01 M ammonium acetate using plain carbon paste electrodes (PCPEs) and the modified carbon paste electrodes (MCPEs). Appropriate quantities of Cu(II), Co(II), Ni(II), Mn(II) and Pb(II) were added to the media to attain a final concentration of 1 – 5 mM, to study the catalytic effect on electrooxidation.

Differential pulse voltammograms were recorded at a scan rate of 10 mVs^{-1} and amplitude of 50 mV. Commercial samples were analyzed by DPV using the standard addition method in 0.01 M ammonium acetate containing 5 mM $Pb(NO_3)_2$.

Sample Preparation

Pharmaceutical preparations analyzed were ALGIDOL (Almirall Prodesfarma, Spain), ASPIRINA – C (Bayer, Portugal), FEMIVIT (laboratorios Effik, Spain) and CALCINATAL (Pfizer, Spain). ALGIDOL was in powder form, 1 g of which was weighed and dissolved in 0.04 M acetic acid (pH 3) to give a stock solution. In addition to ascorbic acid it contained paracetamol, codeine phosphate, and sodium saccharin. ASPIRINA – C, containing sodium carbonate, sodium hydrogen carbonate and acetyl- salicylic acid; and CALCINATAL, containing multiple vitamins and minerals, were in tablet form, which were powdered and taken up in acetic acid as above. In case of ASPIRINA – C excess acetic acid had to be added to neutralize the bases and allowed to stand to expel carbon dioxide. FEMIVIT, containing multiple vitamins and minerals in capsule form, was dissolved in acetic acid overnight. After dissolution, all solutions were filtered to obtain clear solutions.

Two samples of fruit juices, one of pineapple (Compal, Portugal) and one of multiple fruits (Compal, Portugal) were filtered and the filtrate was directly taken up in *supporting electrolyte for analysis*.

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