Simultaneous Anodic Assessment of Ascorbic Acid and Acetaminophen in Unbuffered Solutions

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The simultaneous detection of L-ascorbic acid (AA) and acetaminophen (AC) at a boron-doped diamond electrode (BDDE), in a sodium sulphate supporting electrolyte, using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was investigated. The calibration plots resulting from two distinct series of anodic current peaks versus concentration of AA and AC in single and di-component systems, obtained from CV and DPV data, presented linearity, with very good correlation coefficients. Fouling of the electrode surface was not evident and BDDE showed very reproducible voltammetric signals in all investigated situations. The relative standard deviations of 2 -3 %, high sensitivity values, and low limits of detection evaluated from DPV data in single systems, have been practically recovered in di-component standard and real sample solutions. The anodic assessment with the use of DPV was additionally verified by the simultaneous determination of AA and AC in real samples prepared from pharmaceutical products. Using a very simple supporting electrolyte, neutral unbuffered sodium sulphate solution, the DPV at a BDDE was confirmed as an easily applicable electroanalytical option. Individual and average values corresponding to AA and AC content in an investigated pharmaceutical product were determined by DPV coupled with standard addition method giving results in good accordance with the composition indicated by the supplier.

Keywords: acetaminophen, ascorbic acid, differential pulse voltammetry, unbuffered media, boron-doped diamond electrode

Associated pharmacologically active compounds have received special attention owing to their synergetic effects. Pharmaceutical formulations consisting of ascorbic acid (AA) and acetaminophen (AC) in combination, for example, are frequently commercialized.

Acetaminophen or paracetamol (N – acetyl – *p* – aminophenol), often associated with other active substances, *e.g.* ascorbic acid, caffeine, or phenobarbital, is an analgesic and antipyretic drug extensively employed due to its lack of gastric complications [1-4]. In proper therapeutic dose, acetaminophen is readily transformed [5] into inactive metabolites which are eliminated in the urine [2]. Overdoses of acetaminophen can lead to accumulation of toxic metabolites, which may cause severe and sometimes fatal hepatotoxicity and nephrotoxicity [6].

L-ascorbic acid or Vitamin C (L-threo-hex-2-enono-1,4-lactone) is a water-soluble organic compound of special biological importance, found in natural products and organisms, and in a large number of pharmaceutical and food preparations [7]. AA is known for its reducing properties and for its use as an antioxidant and protective agent in biological systems. Association between ascorbic acid and other biologically active substances, including acetaminophen [8], in pharmaceutical formulations and metabolic fluids is also mentioned [9, 10]. Ascorbic acid is well known to be highly labile, being susceptible to oxidative degradation by the action of oxygen associated with light, alkaline media, heat, the presence of various metal contaminants, or electrochemical oxidation [11].

A variety of several available methods with different detection techniques for the determination of ascorbic acid and acetaminophen in biological fluids, beverages, food, and in pharmaceutical preparations have been reported. Such methods as titrimetry, spectrofluorimetry, spectrophotometry, FTIR-spectrometry, high performance liquid chromatography and others have been applied [12-16].

Acting as electroactive substances, ascorbic acid and acetaminophen have also been electrochemically studied from a mechanistic or analytical perspective [4-8, 14, 21-36], using a range of electrode types and supporting electrolytes. A detailed reaction mechanism describing the electrochemical oxidation of acetaminophen has been given by Kissinger and co-workers [21, 22].

The boron-doped diamond (BDD) is considered an important material for electroanalysis, since it has several electrochemical valuable properties such as a wide potential window in aqueous solution, a low background current and a high stability. These characteristics make it significantly superior to other commonly used electrode materials [37- 42]. The use of boron doped-diamond electrode (BDDE) for an individual investigation of electrochemical behaviour of AA as well as AC in single component systems has been reported [43, 44]. Moreover, our previous investigations [45, 46] treat the application of the BDDE for simultaneous determination of these compounds in Britton-Robinson buffered medium by voltammetry and chronoamperometry.

In this paper we report a new investigation of the simultaneous behaviour and assessment of ascorbic acid and acetaminophen at an unmodified BDDE using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in a very simple and easily accessible neutral unbuffered medium, sodium sulphate solution used as supporting electrolyte. A practical application of the DPV associated with standard addition method developed in circumstance of a neutral sodium sulphate supporting electrolyte was confirmed with good results through the electroanalysis of an UPSA pharmaceutical product.

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Experimental part

The electrochemical data were obtained from CV and DPV measurements. A Metrohm three-electrode cell equipped with a BDDE, a 3 mm-diameter stationary disc embedded in a Teflon rod as working electrode, a platinum foil counter-electrode and a saturated calomel reference electrode (SCE), were used to perform the electrochemical measurements. The commercial diamond electrode supplied by Windsor Scientific Ltd. for electroanalytical use was a mirror-polished doped polycrystalline industrial diamond (microcrystalline; doping degree ~ 0.1% boron) [42, 45, 46]. All the voltammograms were collected using an Autolab PGStat 20 EcoChemie system controlled by a PC running GPES Software version 4.8. Working parameters corresponded to a scan rate between 0.01-0.1 Vs^{-1}, usually of 0.05 Vs^{-1}, a potential range between -0.6 V and + 1.25 V vs. SCE, for CV, and a modulation time of 0.05 s, an interval time of 0.25 s, an initial potential of 0V, an end potential of 1.25 V, a step potential of 0.00405 V, a modulation amplitude of 0.02502 V, and a scan rate of 0.0162 Vs^{-1} for DPV. The working electrode was carefully cleaned, degreased and treated to remove fouling by polishing with alumina powder (0.1-0.3 mm), and finally washed with double-distilled water prior to the start of each measurement series. Each determination was repeated three times, without supplementary cleaning of the electrode between the successive measurements, a short rest period and stirring of the solution between replicates proving sufficient for reproducible recording. The supporting electrolyte was a 0.1 M Na_2SO_4 unbuffered solution, *p*H 7. The substances used were analytical grade Fluka and Merck reagents. The voltammograms were recorded at the stationary electrode and at room temperature (23±1°C). The quiescent solutions were deaerated with argon and maintained under an argon atmosphere. The electroanalytical application of the DPV method coupled with standard addition for simultaneous detection and determination of AA and AC was trialled using Efferalgan tablets (UPSA). AA and AC standard solutions as well as Efferalgan solution were prepared daily, prior to use. The explored concentrations of ascorbic acid and acetaminophen ranged between 0.01-0.1 mM. The samples from pharmaceutical formulation were aqueous solutions prepared by dissolution of Efferalgan effervescent tablets, one tablet per 500 mL. The final working solution volume in the cell was 50 mL and the standard additions, being only very small volumes of concentrated solutions, were carried out without major corrections. All the dilutions concerning investigated standard and real sample solutions were made using unbuffered supporting electrolyte.

Results and discussions

Several preliminary cyclic voltammograms (not presented here) for mixture solution proved the additivity of AA and AC amperometric signals from individual systems.

Figure 1 illustrates two examples of overall cyclic voltammograms (CVs), as first scan, from a large series collected for the mixture of AA and AC standard solutions within the concentration range 0.01-0.1mM. The exemplified CVs, corresponding to irreversible processes, were recorded in a potential range between -0.6 V and + 1.25 V vs. SCE, starting in the anodic direction from 0 V to + 1.25 V vs. SCE. On the forward branch of CV two well defined and separated anodic current peaks corresponding to both AA and AC can be seen around + 0.4 V and + 0.6 V vs. SCE, respectively.

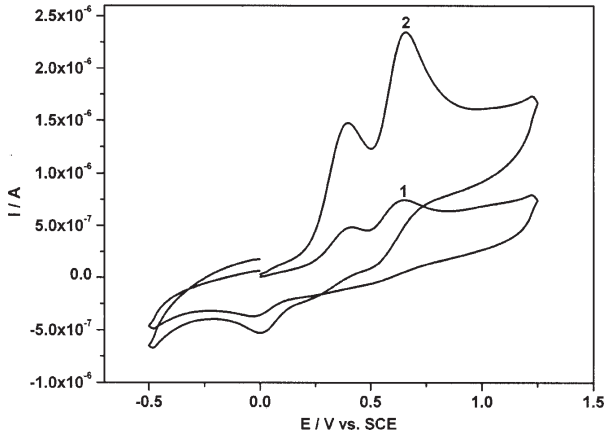

Fig. 1. Cyclic voltammograms (CVs) of AA and AC mixture; 1 – 0.02 mM AA and 0.02 mM AC; 2 – 0.07 mM AA and 0.07 mM AC; supporting electrolyte - 0.1 M Na_2SO_4, pH 7; starting potential 0V vs. SCE; potential range: -0.6 V → + 1.25 → 0 V vs. SCE; scan rate: 0.05 Vs^{-1}

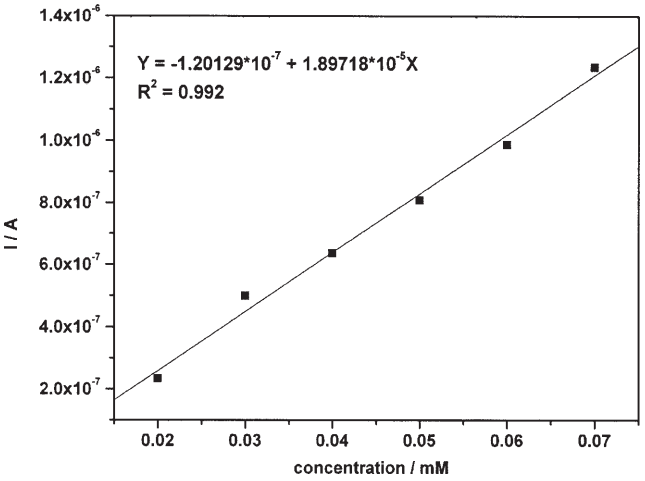

Fig. 2a. Calibration plot (with supporting electrolyte – background current correction) of peak current, I vs. AA concentration (0.01 mM – 0.1 mM) around + 0.4 V vs. SCE (under the conditions mentioned in fig.1)

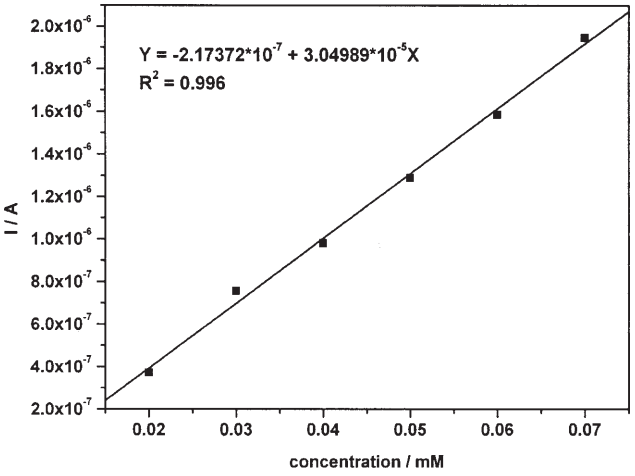

Fig. 2b. Calibration plot (with supporting electrolyte – background current correction) of peak current, I vs. AC concentration (0.01 mM – 0.1 mM) around + 0.6 V vs. SCE (under the conditions mentioned in fig. 1)

The linear calibration plots resulting from cyclic voltammograms are shown in figures 2a and 2b. The dependencies of peak current versus concentration of AA and AC in di-component solution presented very good correlation parameters and confirmed the predominant

control of the anodic step of the individual processes by diffusion. All the characteristics mentioned above offer a useful basis for the simultaneous assessment of the investigated substances by differential pulse voltammetric measurements at BDDE. Several considerations concerning a possible correlation between *p*H changes in adjacent layer of the working electrode and unbuffered or buffered media characteristics can be advanced. In neutral buffered media, explored as phosphate buffer, the anodic current peaks of AA and AC were practically overlapped on the forward anodic branch of CV and very close on the DPV [45]. On the contrary, in acidic buffered Britton-Robinson media, a very good simultaneous detection by two distinct current peaks manifested on CV as well as DPV was obtained. This difference can be attributed to an overall change in the adjacent *p*H condition. A first interpretation involves a general idea regarding the acidic *p*H and the favourable role it plays in giving a good separation of anodic voltammetric peaks. A simple reconsideration of each overall oxidation process regarding AA and AC [8, 21, 22] can be met according to this hypothesis. The anodic oxidations of AA and AC occur as deprotonating processes [8, 21, 22] with local acidulation. In a neutral aqueous buffered medium, such as phosphate buffer, the change of *p*H is suppressed, but in a neutral unbuffered medium, such as sodium sulphate solution, adjacent *p*H evolution occurs as a free acidulation which works favourably towards the separation of the current peaks.

Therefore, a detailed investigation concerning DPV application for detection and determination of AA and AC in single component, di-component standard solutions, and real samples in such neutral unbuffered media has been achieved.

The effect of AA concentration in standard solutions on the optimum anodic response has been evaluated from the series of DPVs presented in figure 3. Sharp anodic current peaks attributed to AA oxidation manifested around + 0.4 V vs. SCE. Calibration plot of anodic peak current versus AA concentration (inset of fig. 3) was linear, with a high sensitivity value of 4.40 μA mM^{-1}, and a very good correlation coefficient.

Figure 4 shows similarly a series of DPVs regarding the effect of AC concentration in the range 0.01 mM–0.1 mM using standard solutions in a sodium sulphate supporting electrolyte. The well defined current peaks corresponding to AC anodic oxidation placed around + 0.6 V vs. SCE. Linear plot of anodic peak current versus AC concentration (inset of fig. 4) also showed a good correlation coefficient and a sensitivity value of 5.77 μA mM^{-1}.

In line with the presence of both compounds in the mixture solutions two distinct current peaks can be observed in the DPVs presented in figure 5.

The corresponding peak potentials were shifted progressively with increase of concentrations, as it was for individual systems (figs. 3 and 4), more markedly for AC, possibly due to the progressive adjacent *p*H diminution.

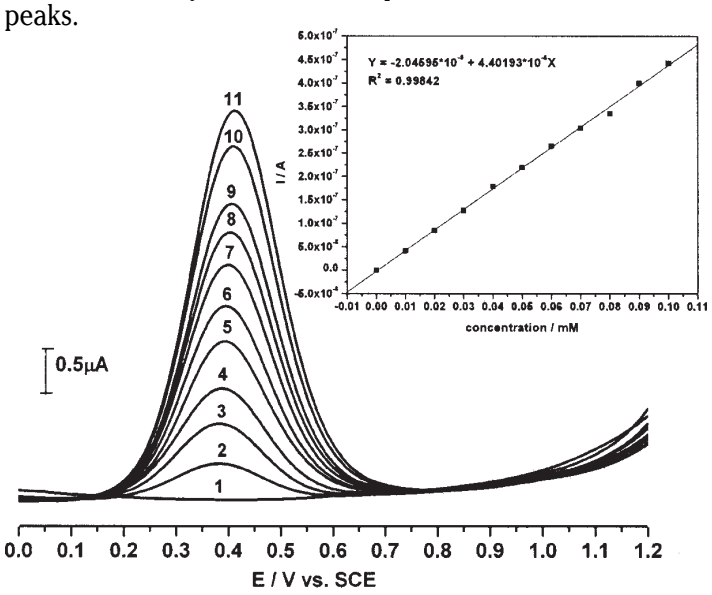

Fig. 3. Differential pulse voltammograms (DPVs); effect of AA concentration; 1 – supporting electrolyte - 0.1 M Na_2SO_4 *p*H 7; 2 – 0.01 mM AA; 3 – 0.02 mM AA; 4 – 0.03 mM AA; 5 – 0.04 mM AA; 6 – 0.05 mM AA; 7 – 0.06 mM AA; 8 – 0.07 mM AA; 9 – 0.08 mM AA; 10 – 0.09 mM AA; 11 – 0.1 mM AA; *inset*: Calibration plot (with supporting electrolyte – background current correction) of peak current, I vs. AA concentration around + 0.4 V vs. SCE

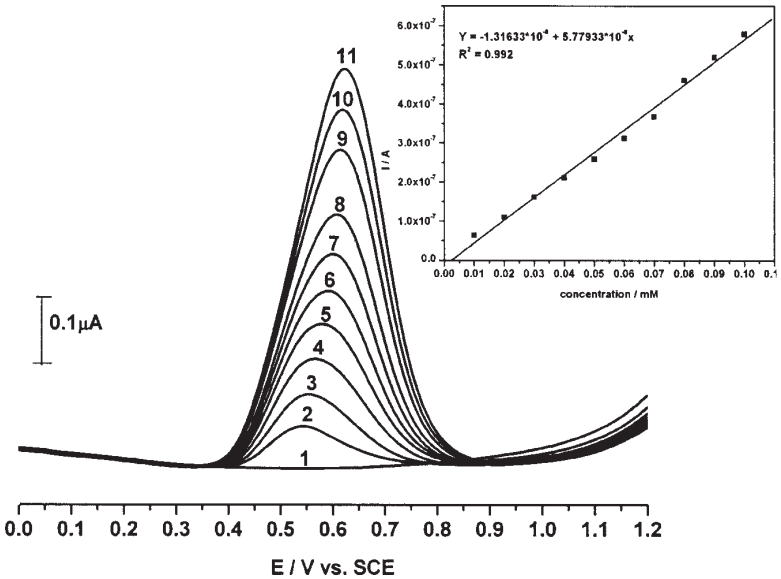

Fig. 4. Differential pulse voltammograms (DPVs); effect of AC concentration; 1 – supporting electrolyte - 0.1 M Na_2SO_4 pH 7; 2 – 0.01 mM AC; 3 – 0.02 mM AC; 4 – 0.03 mM AC; 5 – 0.04 mM AC; 6 – 0.05 mM AC; 7 – 0.06 mM AC; 8 – 0.07 mM AC; 9 – 0.08 mM AC; 10 – 0.09 mM AC; 11 – 0.1 mM AC; inset: Calibration plot (with supporting electrolyte – background current correction) of peak current, I vs. AC concentration around + 0.6 V vs. SCE

Attribution of this feature to the trend of the adjacent *p*H, leading to a degree of free and unbuffered acidification, is in accordance with our discussed hypothesis which considered the role of the local *p*H on the well separation of anodic current peaks.

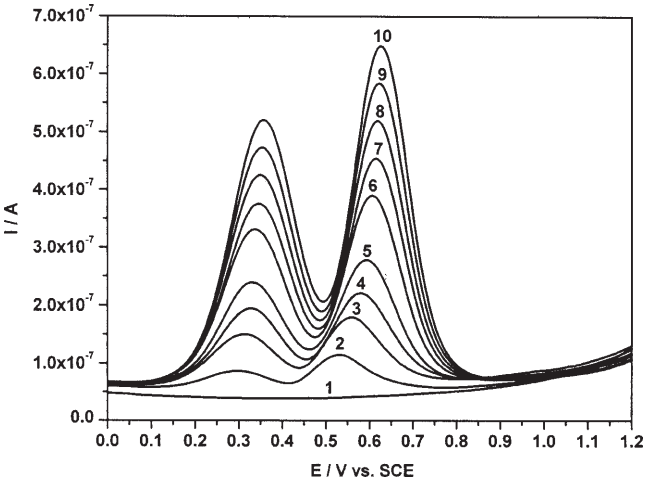

Fig. 5. Differential pulse voltammograms (DPVs) of AA and AC mixture; AA and AC added in increasing concentration; 1 – supporting electrolyte - 0.1 M Na_2SO_4 pH 7; 2 – 0.01 mM AA and 0.01 mM AC; 3 - 0.02 mM AA and 0.02 mM AC; 4 - 0.03 mM AA and 0.03 mM AC; 5 - 0.04 mM AA and 0.04 mM AC; 6 - 0.06 mM AA and 0.06 mM AC; 7 - 0.07 mM AA and 0.07 mM AC; 8 - 0.08 mM AA and 0.08 mM AC; 9 - 0.09 mM AA and 0.09 mM AC; 10 - 0.1 mM AA and 0.1 mM AC

A linear calibration plot of anodic peak current versus AA concentration in the mixture solution (fig. 6 a) was obtained with a very good correlation coefficient and a sensitivity of 4.67 μA mM^{-1}. Figure 6 b depicts two linear calibration plots of anodic peak current versus AC concentration in the di-component system presenting very good correlation parameters and sensitivities of 5.91μA mM^{-1} (curve 1 being obtained without any current correction) and 5.32 μA mM^{-1} (curve 2 resulted under conditions of an AA remnant current correction), respectively. The AA influence on AC oxidation can be described through a residual current attributed to AA and noticed in the optimal potential range corresponding to AC detection. Therefore, a correction based on the AA remnant current has been applied. In order to make this correction, the current values for AA read around + 0.6V vs. SCE

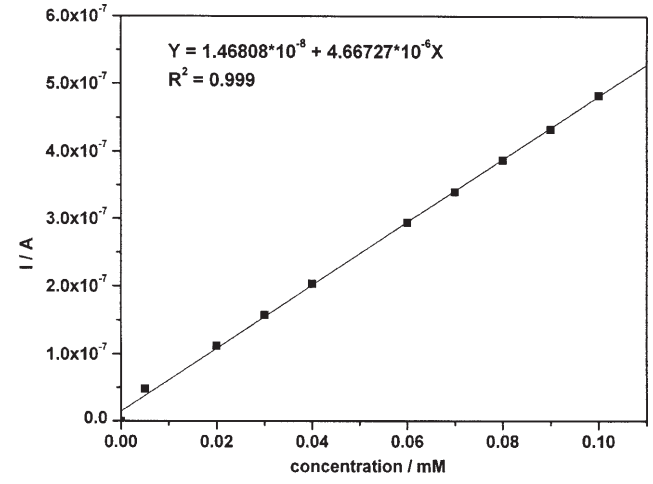

Fig. 6a. Calibration plot (with supporting electrolyte - background current correction) of peak current, I vs. AA concentration in mixture solution around + 0.4 V vs. SCE (under the conditions mentioned in fig. 5).

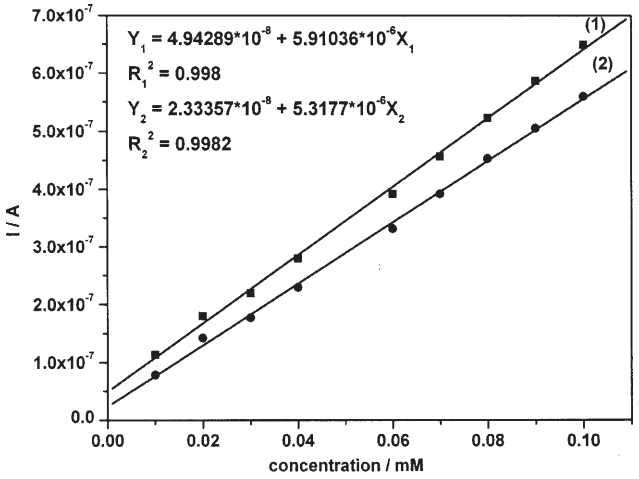

Fig.6b. Calibration plots of peak current, I vs. AC concentration in mixture solution around + 0.6 V vs. SCE (under the conditions mentioned in figure 5): (1) – without background current correction; (2) – with subtraction of AA current read (in individual case) around + 0.6 V vs. SCE, as an approximated correction for AC.

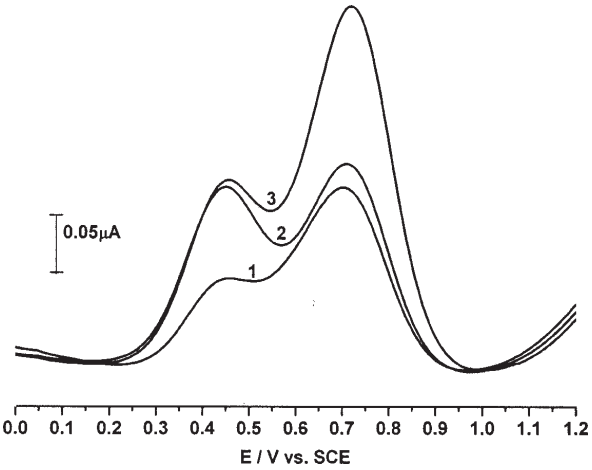

Fig. 7. Differential pulse voltammograms (DPVs); 1) 0.5/50 dilution of Efferalgan initial solution in supporting electrolyte - 0.1 M Na_2SO_4 *p*H 7; 2) 0.5/50 dilution of Efferalgan initial solution in supporting electrolyte and AA addition, 0.02 mM AA final supplementary concentration; 3) 0.5/50 dilution of Efferalgan initial solution in supporting electrolyte, AA and AC addition, 0.02 mM AA and 0.04 mM AC final supplementary concentrations

(fig. 3) were subtracted from those for AC in the mixture solution case (fig. 5).

The utility of DPV method for the assessment of AA and AC in single component and di-component systems was suggested by the relatively high sensitivities, relative standard deviations between 2 and 3%, and also by low values of limit of detection (LOD). In single component solutions, LOD was estimated to be 1.31 μM for AA and 1.5 μM for AC, and in di-component solutions, 1.59 μM for AA and 1.72 μM for AC, respectively. The potential usefulness of the method was verified by practical DPV data from the simultaneous determination of AA and AC in a particular pharmaceutical formulation. For this purpose, aqueous solutions obtained from Efferalgan tablets (UPSA) were used as real samples.

Figure 7 presents an example involving the simultaneous determination of AA and AC in an Efferalgan real sample solution. The tested real sample was prepared under the conditions mentioned in the experimental part and using a

2.9650 g Efferalgan tablet. In order to obtain concentrations of 0.02 mM AA and 0.04 mM AC in the final real sample solution, both ascorbic acid and acetaminophen standard solutions were added in very small volumes.

The shift of the potentials corresponding to current peaks for dilute real sample solution with various standard additions suggested certain secondary effects comparatively with similar data obtained in individual and mixture systems of dilute solutions produced from standards. These matrix effects, which can be attributed to particular ingredients present in the tablets, were insignificant in their impact on the quantitative evaluation of AA and AC in mixture.

Using the DPV technique coupled with standard addition method, the average contents of the investigated compounds in the example case of the UPSA product were 197.6 mg AA / tablet and 326.5 mg AC / tablet. The data resulted from standard addition method for five investigated Efferalgan tablets, with an average weight of 2.9258 g, gave average values of 198.4 mg AA / tablet and 328.2 mg AC / tablet. According to the general UPSA product specification, each single tablet should contain 200 mg AA and 330 mg AC.

Conclusions

A new electroanalytical option for the simultaneous assessment of ascorbic acid and acetaminophen at an unmodified boron-doped diamond electrode using as supporting electrolyte an easily accessible and simple unbuffered sodium sulphate solution of *p*H 7 has been achieved using differential pulse voltammetry.

Cyclic voltammograms of AA and AC recorded in di-component solutions presented two well defined separated current peaks attributed to anodic oxidation of AA or AC and disposed around + 0.4 V vs. SCE (for AA) and + 0.6 V vs. SCE (for AC), respectively. The good separation of anodic current peaks was attributed to adjacent *p*H evolution that occurred as free acidulation in neutral unbuffered media, as distinct from behaviour in a neutrally buffered solution in which any pH changes were suppressed.

The simultaneous determination of AA and AC in real samples obtained from a particular UPSA pharmaceutical product has also been accomplished using the elaborated method as differential pulse voltammetry at a BDDE in unbuffered neutral media coupled with standard addition. The average contents determined for a series of Efferalgan (UPSA) real sample solutions prepared from five tablets were in very good agreement with contents specified by the producer for one tablet.

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