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The use of carbon paste electrode in the direct voltammetric determination of tryptophan in pharmaceutical formulations

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Abstract

A simple, fast and sensitive method is proposed for Tryptophan (Trp) determination in pharmaceutical formulations containing other non-electroative aminoacids, vitamins and hydroxycobalamines. Optimized conditions for differential pulse voltammetry allowed the determination of Trp with detection limit of 1.7 μM in 2–30 mM linear dynamic range ($n = 7$; $r = 0.999$), at a bare, non-treated carbon paste electrode. Ascorbic acid interference was evaluated and eliminated by choosing a suitable baseline for determination of peak currents for Trp.

Keywords: Tryptophan; Differential pulse voltammetry; Carbon paste electrode

1. Introduction

Tryptophan (Trp) is an essential amino acid for humans and herbivores scarcely present in vegetable products. It is sometimes added to dietary and feed products as a food fortifier and to pharmaceutical formulations in order to correct possible dietary deficiencies. It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance. Trp is also a precursor of the neurotransmitter serotonin.

Methods for the determination of Trp are mainly based on HLPC [1–6] and spectrophotometric procedures [7–15]. Most of the spectrophotometric methods involve laborious and slow procedures with the modification of tryptophan by numerous reagents. Trp has also been analyzed by HPLC with a pre-column derivatization and fluorescence detection with excitation at 330 nm and emission at 445 nm [1] or post-column derivatization with chemiluminescence detection of the decomposition of hydrogen peroxide catalyzed by copper(II)–Trp complex [2] in order to improve sensitivity, or without derivatization by using its absorbance at ultraviolet region [3], fluorescence [4] or electrochemical properties [5,6]. However, the chromatographic separation is often complex, tedious and time-

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consuming. For these reasons there is an interest in developing faster, simpler and less expensive procedures for Trp analysis.

Few methods are based on electroanalytical methods using mercury [16], carbon paste [17–21] and graphite-metacrylate composite [22,23] electrodes. Carbon paste has been used with chemical modifiers. Sugawara et al [17] relate the possibility of the determination of Trp and some other amino acids at a level of 10^{-6} M by a chemical modified carbon paste electrode with copper(II) cyclohexylbutyrate and reverse linear sweep voltammetry after reduction of Cu(II). Differential pulse voltammetry (DPV) using modified carbon paste electrode has been also reported [18,19] for the direct determination of Trp. In these studies linear dynamic ranges between 9.8 and 98 μM and a limit of detection of 1.5 μM were observed [18] and sometimes there is a disadvantage that the method needs a medium exchange procedure that resulted in a linear range from 9.8 to 49 μM with limit of detection of 0.3 μM [19]. An indirect adsorptive stripping voltammetric method for determining Trp was developed by Zoulis et al. [20], which is based on the enzymatic cleavage of the amino acid to indole and the pre-concentration of indole at the carbon paste electrode. This method also requires a medium exchange procedure and a 140 min incubation time. Another adsorptive stripping voltammetric determination of Trp was developed by Wang et al [21] by using an electrochemically pre-treated carbon paste electrode. Although this method presents better sensitivity (linear range: 39–490 nM), the time of chemical pre-treatment of the electrode is long (10 min). Recent studies have been proposed using multivariate calibration methods for cyclic simultaneous voltammetric determination of oxidizable amino acids, including Trp, at a graphite–methacrylate composite electrode [22,23]. This methodology is applicable to complex matrix compositions, in which the accuracy of the analysis is not as crucial as its speed.

In this work, the possibility of the use of a direct differential pulse voltammetric determination of Trp in pharmaceutical formulations using bare carbon paste electrode (75% graphite, 25% nujol) is described in a rapid, inexpensive and sensitive procedure. The method has been successfully applied to commercial samples with different formulations.

Differential pulse voltammetry (DPV) is an electroanalytical technique for solid and mercury film electrodes based on a waveform resulting from the sum of a staircase with a pulse train. Just prior to the application of the small-amplitude pulse a current sample is taken and a second sample is taken at the end of the pulse. The resulting DPV voltammogram is a plot of the difference between the two current samples versus the applied potential [24,25].

2. Experimental

2.1. Reagents

All chemicals were used as received. 1.0 mM Trp (Synth, Brazil) stock solutions, Britton-Robinson buffer (pH 7.4, 155 mM phosphate, 155 mM H_3BO_3, 245 sodium acetate, $\mu = 0.5$ M) and phosphate buffer (pH 7.4; 224 mM phosphate, $\mu = 0.5$ M) were used. All solutions were prepared using twice distilled water in a quartz distillator.

Commercial tablet formulations containing Trp were pulverized in a mortar and suitable mass partially dissolved in the buffer was treated for 40 min in an ultrasonic cleaner apparatus in order to dissolve completely. Liquid samples were directly diluted in the buffer. All the samples were purchased in drugstores.

2.2. Carbon paste electrode (CPE)

CPE without modification has been prepared by mixing 75% (m/m) Acheson-38 graphite powder (Fisher), and 25% (m/m) mineral oil, Nujol (Aldrich—density 0.838 g/ml). The mixture was homogenized in a mortar for 20 min.

The resulting paste was inserted in a 1.0 ml plastic syringe and a copper wire was used for electric contact.

2.3. Apparatus

All the measurements were performed in a potentiostat/galvanostat 264A EG&G (PAR-C) coupled to an X–Y Omnigraph 100 recorder (Huston Instruments). A 10 ml cell was used with a three electrode configuration. The working electrode was a CPE prepared as above, with area ca. 0.28 cm^2, whose surface was renewed before each measurement on a bond paper. The auxiliary electrode was a platinum plate, 1.0×0.5 cm, and a homemade Ag/AgCl as the reference electrode.

A homemade glassy carbon electrode (GCE), was constructed by connecting a 3 mm diameter glassy carbon piece to a copper wire with silver epoxy (EPO-TECH 410-E, Epoxy Technology, USA). This unit was then sealed in a 5 mm i.d. glass tube with an epoxy resin, and was used as working electrode in the preliminary studies.

2.4. Procedure

A working potential window was first established using cyclic voltammetry (CV) and the effect of scan rate was also evaluated. Using the best conditions an analytical curve was obtained with this technique in phosphate buffer.

In the potential window defined by CV, the best pulse amplitude was determined for measurements using differential pulse voltammetry (DPV). A scan rate of 20 mV s^{-1} was fixed. Analytical curves were obtained under the conditions established here in phosphate buffer.

Standard addition procedure was used for determining Trp content in the commercial samples, under the conditions defined for DPV.

The results were compared with the spectrophotometric procedure described by Verma et al. [12] which is based on a specific reaction for compounds that present indolic groups in their structures. The method involves an indirect determination of Trp after reaction with HNO_2 and monitoring the absorbance at 400 nm.

The effect of the presence of ascorbic acid (AA) was investigated using solutions containing 15 μM Trp and 0 to 162 μM AA, in DPV under the same conditions of Trp determination.

The peak current values were determined by constructing a tangent to the shoulders of each respective peak (or to a shoulder at the more anodic potential, in case of standard addition curve for determination of Trp in samples containing AA) and measuring the perpendicular height between the tangent and the maximum of the peak.

3. Results

3.1. Voltammetric studies

In this work the idea was to propose a simple and fast method for the determination of Trp. Thus a preliminary study has been developed in order to compare the response of CPE and glassy carbon (GC) electrodes both unmodified. For Trp at μM concentration level very low sensitivity and resolution was observed for GC, pointing to the CPE as the best choice for the determination of this analyte in the further studies. The differences should be related with the higher active area of the CPE when compared with GC.

For CV, the potential window ranging between 0.00–1.20 V (versus Ag,AgCl) and scan rate from 100 to 500 mV s^{-1} was investigated in phosphate buffer. Better CPE sensitivity for Trp was found at 200 mV s^{-1} scan rate in the 0.00–1.00 V potential window. The parameters of the analytical curve obtained under the optimized CV conditions are presented in Table 1.

The effect of pulse potential height was evaluated under the CV optimized potential window and the scan rate fixed at 20 mV s^{-1} in phosphate buffer as support electrolyte. Better CPE performance was found at a pulse amplitude of 50 mV.

The comparison of phosphate and Britton–Robinson buffers was evaluated by DPV at 20 mV s^{-1} under the pulse amplitude and potential window described above. Higher CPE sensitivity and peak definition for Trp at 5 μM level was observed in phosphate buffer (88.9 mA M^{-1}) in relation to Britton–Robinson buffer (53.8 mA M^{-1}). Fig. 1 represents typical DPV curves for Trp in phosphate and Britton–Robinson buffers. Parameters for the analytical curve in DPV for Trp under CPE's best performance are in Table 1.

Table 1
Results for analytical curves ($y = a + bx$) obtained under CV and DPV optimized conditions

Technique	a/μA	Sensitivity/mA M^{-1}	Linear range/μM	Limit of detection[a]/μM	r ($n = 7$)[b]
CV	0.105 ± 15.0	8.15 ± 0.27	29.1–82.6	4.66	0.997
DPV	4.70 ± 2.60	84.0 ± 1.8	2.06–30.0	1.66	0.999

[a] $3 \times$ S.D./angular coefficient [27].
[b] Linear correlation coefficient.

The differences observed in these two buffers should be related to their composition and ionic strength, which can cause changes in the electron transfer kinetics in irreversible reactions and the peak shape, resulting in low definition [24].

Using DPV under the optimized conditions discussed above, a linear dynamic range between 2–30 μM was observed for Trp, with the detection limit of 1.66 μM. Above 30 μM the response is not linear probably due to the adsorption of the Trp in the electrode surface as described by Brabec and Mornstein [26]. The equation for the linear relationship is $y = (4.70 \pm 2.60\ \mu A) + (84.0 \pm 1.8\ mA\ M^{-1})x$, with linear correlation coefficient $r = 0.999$ ($n = 7$). The limit of detection obtained earlier [18,19] are in the same concentration level observed here, however the present method allows a faster and easier procedure.

3.2. Determination of Trp in pharmaceutical samples

Since the sensitivity of CPE for Trp in DPV is 10 times higher than in CV, DPV was used in the determination of this analyte in pharmaceutical samples.

The results for the standard addition procedure using the DPV proposed method applied to these samples are shown in Table 2. Typical sensitivities from 60–97 mA M^{-1} were observed in the analytical curves.

The content of Trp in such samples determined by the present method using DPV at CPE are in good agreement with the label specifications and those determinated by indirect spectrophotometric method as shown in Table 3. The main advantage is that the present method is faster and easier than the spectrophotometric procedure, which takes 30 min just for the derivatization step.

The presence of AA (sample 2), that oxidizes at less anodic potential than Trp, causes changes in the profile of the sample voltammogram at less anodic potential range than that where Trp oxidation peak appears.

When the current is determined by a shoulder to shoulder extrapolation procedure, a negative interference of AA over Trp peak is observed. Fig. 2 presents the effect at different molar ratios

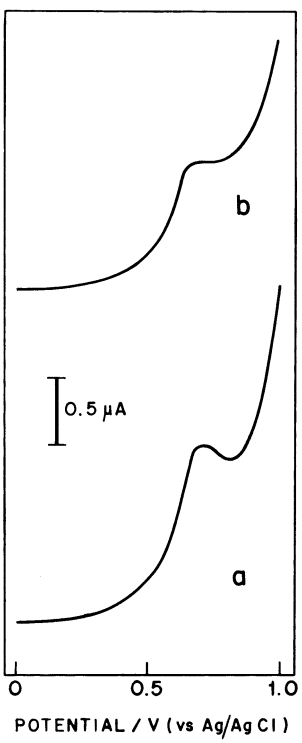

Fig. 1. Differential pulse voltammograms for 5 μM Trp in phosphate buffer pH 7.4 (a) and Britton–Robinson buffer pH 7.4 (b) at CPE. Conditions: scan rate = 20 mV s^{-1}, pulse amplitude: 50 mV, potential window: 0.00–1.00 V (vs. Ag/AgCl).

Table 2
Experimental parameters for standard addition curves for Trp determination in pharmaceutical samples

Sample[a]	Composition	[Trp]/mM	r[a]
Sample 1	Buclizine chloride (25 mg)[b] L-Lysine chloridrate (200 mg)[b] Pyridoxine chloridrate (20 mg)[b] Cyanocobalamin (50 μg)[b] Starch[c]	7.41 ± 0.01	0.999
Sample 2	Retinol acetate (5000 IU)[b] Thiamine chloridrate (10 mg)[b] Riboflavin (10 mg)[b] Ascorbic acid (25 mg)[b] Tocopherol acetate (20 mg)[b]	7.17 ± 0.10	1.00
Sample 3	L-phosphotreonine (10 mg)[d] L-glutamine (60 mg)[c] L-phosphoserine (40 mg)[c] L-arginine (100 mg)[c] Hydroxycobalamin (500 μg)[c]	9.26 ± 0.22	0.998
Sample 4	Buclizine chloridrate (10 mg)[c] L-Lysine (300 mg)[c] Cysteine chloridrate (2 mg)[c] Pyridoxine chloridrate (20 mg)[c] Cyanocobalamine (50 μg)[c] Sodium saccharinate[d] Citric acid[d]	9.17 ± 0.07	1.00

[a] Purchased in drugstores.
[b] In one tablet.
[c] In a 10 ml sample.
[d] Quantity not labeled.

AA/Trp. This interference becomes nearly constant from molar ratios AA/Trp over 1.0. However when the current is measured using a baseline tangent to the more anodic shoulder it can be partially eliminated without sample treatment, as shown in Fig. 2. Up to a mol ratio AA/Trp = 0.5, it is possible to measure the current free of AA interference.

An example of the baseline extrapolation used in the presence and absence of AA is presented in Fig. 3. This procedure, in association with the standard addition method, makes possible the Trp determination in the concentration levels of both analytes present in sample 2 here investigated, in which the ratio AA/Trp = 1.16.

The simultaneous presence of Trp and AA is not common in pharmaceutical formulations. A search in the Vade-Mécum Catalog [28] revealed that for 17 formulations containing Trp, only three products presented both analytes in the label specification, including sample 2. Other two formulations were not analyzed and presents mol ratios of 7.54 and 232. Therefore the present

Table 3
Results for determinations of tryptophan in pharmaceutical samples

Sample	Tryptophan content			Error[a]/%
	DPV[b]	UV[c]	Labeled	
Sample 1	20.4 ± 0.1^{d}	20.5 ± 0.7^{d}	20[d]	−0.5
Sample 2	24.7 ± 0.3^{d}	24.3 ± 0.1^{d}	25[d]	+1.6
Sample 3	18.5 ± 0.4^{e}	18.2 ± 0.1^{e}	20[e]	+1.6
Sample 4	13.4 ± 0.1^{e}	13.3 ± 0.1^{e}	9.8[e]	+0.8

[a] Error of proposed method in relation to the validation method.
[b] DPV proposed method.
[c] Spectrophotometric method of validation.
[d] Solid sample: mg/tablet.
[e] Liquid sample: mM.

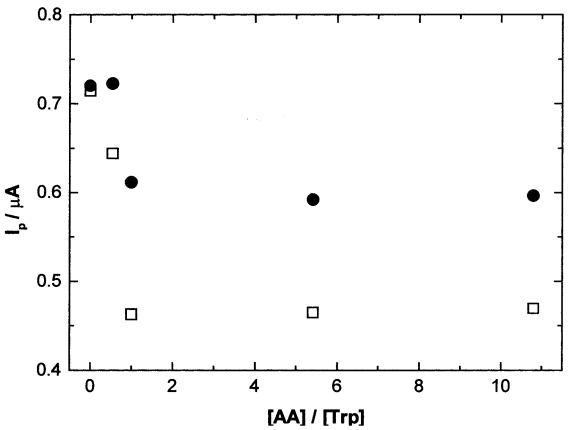

Fig. 2. Interference of ascorbic acid (AA) over anodic mean current ($n = 6$) peak for 15 μM Trp in phosphate buffer pH 7.4 at CPE. Other experimental conditions as in Fig. 1. □ shoulder to shoulder; ● tangent to more anodic shoulder.

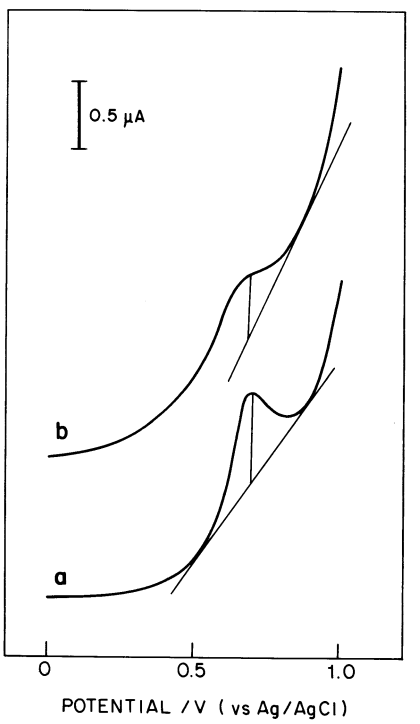

Fig. 3. Different baseline extrapolation used in differential pulse voltammograms for determination of the current of 7 μM Trp at CPE in the (a) absence (sample 1) and (b) presence (sample 2) of ascorbic acid. Conditions: scan rate = 20 mV s^{-1}, pulse amplitude: 50 mV, potential window: 0.00–1.00 V (vs. Ag/AgCl) in phosphate buffer pH 7.4.

method is useful for the most formulations containing Trp.

It is important to note that the baseline extrapolation procedure gives a Trp content very close to the spectrophotometric value even in the presence of AA, with low standard deviations as presented in Table 3. If the Trp content in sample 2 is determined by the 'shoulder to shoulder' baseline a value of 16.3 ± 0.2 while 24.7 ± 0.3 mg/tablet is obtained with the proposed tangent baseline.

4. Conclusions

The DPV proposed method at CPE without treatment and modification allows the determination of tryptophan at a μM level in the presence of non-eletroative aminoacids and the other common constituents of pharmaceutical formulations such as hydroxycobalamine and vitamins (A, B_1, B_2, B_6, B_{12} and E).

The negative interference of AA over the anodic current peak of Trp can be solved by an adequate methodology of measurement of oxidative current of this aminoacid and the use of standard addition method.

The advantage of the proposed method is the use of a fast, inexpensive and sensitive procedure for the determination of tryptophan in pharmaceutical formulations.

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