PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

DETERMINAÇÃO SIMULTÂNEA DE ÁCIDO ASCÓRIBICO, ÁCIDO ÚRICO E DOPAMINA COM ELETRODO DE PASTA DE NANOTUBOS DE CARBONO

SIMULTANEOUS DETERMINATION OF ASCORBIC ACID, URIC ACID, AND DOPAMINE ON CARBON NANOTUBE PASTE ELECTRODE

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Received on March 19, 2024; received as revised form June 01, 2024; accepted June 12, 2024.

RESUMO

Introdução: Ácido ascórbico (AA), ácido úrico (AU) e dopamina (DA) desempenham papéis cruciais no metabolismo humano. Estas substâncias coexistem em fluidos biológicos e seus níveis estão diretamente associados à diversas patologias. Um problema encontrado na detecção eletroquímica direta e simultânea de AA, AU e DA é que estas espécies apresentam potenciais de oxidação muito próximos na maioria dos materiais eletródicos, o que leva a uma sobreposição da resposta voltamétrica. **Objetivo:** Determinar a concentração de AA, AU e DA em amostras de água natural sobre eletrodo de pasta de nanotubos de carbono (EPNTC). **Métodos:** Todas as medidas voltamétricas foram registradas num potenciostato/galvanostato μAutolab (Metrohm) conectado a uma célula eletroquímica de três eletrodos: trabalho, referência (Ag/AgCl, $KCl_{3,0M}$) e auxiliar (platina). O eletrodo de trabalho foi construído em nosso laboratório. Para o preparo da pasta de nanotubos de carbono foram utilizadas as seguintes proporções: 50% NTC + 50% óleo mineral e 65% NTC + 35% óleo mineral. As técnicas voltamétricas: cíclica (VC) e de pulso diferencial (VPD) foram utilizadas para o estudo eletroanalítico. **Resultados:** As faixas lineares para determinação simultânea de AA, AU e DA pela DPV foram: 0,45 – 1,0 mM, 50 – 200 μM e 10 – 90 μM, respectivamente. Os limites de detecção (LODs) para o AA, DA e AU foram: 7,97 mM; 8,57 μM e 5,95 μM, respectivamente. Os desvios padrão relativos (RSD) foram de 4,6; 2,8 e 1,6% para 0,45 mM de AA, 50 μM de DA e 50 μM de AU. **Discussão:** O mecanismo de oxidação do: AA, AU e DA é um processo que envolve dois elétrons e 2 prótons. A detecção eletroquímica de DA na presença de altas concentrações de AA sobre eletrodos de carbono torna-se difícil devido à oxidação catalítica do AA pela DA. Para as três moléculas: AA, AU e DA, observa-se que as correntes de pico de oxidação aumentaram com o aumento da concentração. **Conclusões:** O EPNTC permitiu a separação dos picos de oxidação da mistura ternária de AA, AU e DA por voltametria cíclica e quando associado à DPV permitiu a determinação simultânea quantitativa de AA, AU e DA.

Palavras-chave: *Catecolaminas; Voltametria cíclica; Voltametria de pulso diferencial.*

DOI: 10.52571/PTQ.v21.n47.2024_01_JULIAO_pg_01_09.pdf

ABSTRACT

Background: Ascorbic acid (AA), uric acid (UA), and dopamine (DA) play crucial roles in human metabolism. These substances coexist in biological fluids, and their levels are directly associated with various pathologies. A significant problem encountered in the direct and simultaneous electrochemical detection of AA, UA, and DA is that these species present very close oxidation potentials on most electrode materials, leading to an overlap in the voltammetric response. **Aim:** The main goal of this work was to determine the concentration of AA, UA, and DA in a natural water sample on a carbon nanotube paste electrode (CNTPE). **Methods:** All voltammetric measurements were performed on a μAutolab potentiostat/galvanostat (Metrohm) connected to an electrochemical cell of three electrodes: working, reference (Ag/AgCl, $KCl_{3.0M}$), and auxiliary (platinum). The working electrode was handmade in our laboratory. The following proportions were used to prepare the paste: 50% CNT + 50% mineral oil and 65% CNT + 35% mineral oil. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used for the electroanalytical study. **Results:** The linear ranges for the simultaneous determination of AA, DA, and UA by DPV were: 0.45 – 1.0 mM, 50 – 200 μM, and 10 – 90 μM, respectively. The LODs of the proposed method for AA, DA, and UA were: 7.97 mM; 8.57 μM, and 5.96 μM, respectively. The relative standard deviations (RSD) were 4.6, 2.8, and 1.6% for 0.45 mM of AA, 50 μM of DA, and 50 μM of UA. **Discussion:** The oxidation mechanism of: AA, UA, and DA is a process that involves two electrons and 2 protons. Electrochemical detection of DA in the presence of high levels of AA on carbon-based electrodes becomes difficult due to the catalytic oxidation of AA by DA. For the three molecules, AA, UA, and DA, it is observed that the oxidation peak currents increased with increasing concentration. **Conclusions:** CNTPE allowed the separation of the oxidation peaks of the ternary mixture of AA, UA, and DA by cyclic voltammetry and, when associated with DPV allowed the simultaneous quantitative determination of AA, UA, and DA.

Keywords: *Catecholamines; Cyclic voltammetry; Differential pulse voltammetry*.

1. INTRODUCTION:

Ascorbic acid (AA), uric acid (UA), and dopamine (DA) play crucial roles in human metabolism. These substances coexist in biological fluids (e.g., blood and urine), and their levels are directly associated with various pathologies. Thus, the individual or simultaneous determination of these molecules is an essential issue in biomedical chemistry and pathological and diagnostic research. A significant problem encountered in the direct and simultaneous electrochemical detection of AA, AU, and DA is that they present very close oxidation potentials on most electrode materials, leading to an overlap in the voltammetric response (Oliveira, 2012).

Dopamine (DA) (Fig. 1) is an endogenous neurotransmitter belonging to the catecholamine family, which plays an essential role in the central nervous system (CNS) and has regulatory properties for hormonal, renal, and cardiovascular functions. In synthesizing catecholamines from the amino acid tyrosine, dopamine is the metabolic precursor of norepinephrine and adrenaline, which act on specific receptors in the CNS, mesenteric, renal, and coronary vessels.

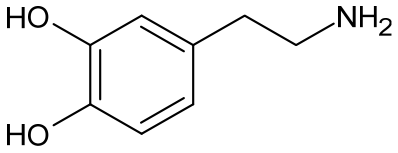

Figure 1. Structure of the dopamine molecule. Source: the authors.

Abnormal amounts of this substance in the human body are directly related to various brain functioning disorders, such as attention deficit, hyperactivity, mood disorders, and poor memory formation, as well as neurodegenerative diseases such as Parkinson's and schizophrenia. It is observed, therefore, that AD has great applicability in the medical field and is also widely used in hospital emergency units for the treatment of shock (cardiogenic, hemorrhagic, and septic) and severe hypotension, as it acts by increasing blood pressure artery and blood flow pumped by the heart (Oliveira, 2012).

Ascorbic acid (AA) or vitamin C is the common name for 2,3-enediol-L-gulonic acid, a notable antioxidant soluble in water and absolute ethanol, in the form of a white or yellowish solid, with a taste similar to the juice of an orange, being found in a wide variety of foods and supplement formulations. Humans cannot produce AA without the enzyme L-glucolactone oxidase, which

converts glucose into AA (Lisboa, 2015). In nature, vitamin C or AA is found in two forms: reduced (commonly known as ascorbic acid) and oxidized (dehydroascorbic acid) (Fig. 2). The oxidation-reduction reaction occurs as follows: removal of two atoms of hydrogen from the AA molecule (oxidation) and addition of two hydrogen atoms recomposing the AA (reduction).

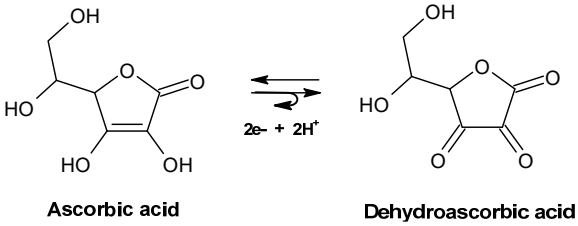

Figure 2. Mechanism of the ascorbic acid oxidation-reduction reaction. Source: the authors.

the main product of the reaction of purines (adenine and guanine). It can also be found in some foods, such as meat, crustaceans, cereals, peanuts, and preserved foods; contributing to the increase in UA in the human body due to the ingestion of these foods. Excess UA in the human body can cause diseases such as gout, nephrolithiasis, and Lesch-Nyhan syndrome, which can be diagnosed due to high concentrations of UA in urine (hyperuricosuria) and blood (hyperuricemia).

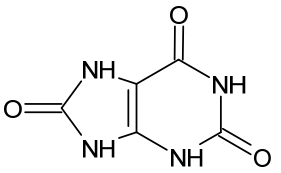

Figure 3. Structure of the uric acid molecule. Source: the authors.

As it is a water-soluble vitamin, it does not accumulate in the body for prolonged periods, being easily excreted through urine, hence the need for its continuous supply through the diet. This vitamin participates in multiple biological functions, being a cofactor of enzymes involved in the post-translational hydroxylation of collagen, the most abundant protein in higher animals and the main constituent of different types of joint tissue connective, giving them various degrees of flexibility and resistance, participates in the biosynthesis of carnitine, a nutrient involved in lipid metabolism, in the conversion of the neurotransmitter dopamine to norepinephrine, in peptide amidation and tyrosine metabolism and also in the absorption of dietary iron, due to the reducing capacity of the ferric form (Fe^{3+}) to the ferrous form (Fe^{2+}) of iron by vitamin C, and also highlight the important role as a dietary antioxidant.

A lack of this nutrient is associated with the appearance of anemia, gum disease, gingivitis, epistaxis, quickly appearing bruises, decreased tissue healing capacity, hair weakening, rough and dry appearance of the skin, arthralgias and swelling of the joints, weakening of tooth enamel, immune deficits, possible weight gain due to a slower metabolism, with severe deficiency being associated with the emergence of Scurvy, a disease that mainly affects groups at risk of malnutrition, such as the elderly. Daily 10 mg of vitamin C can prevent this disease (Diniz, 2015).

Uric acid (UA) is a weak acid poorly soluble in water (6 mg/100 mL), Figure 3. It results from the metabolism of nitrogen within the human body,

This compound is almost eliminated in the urine, in daily amounts ranging from 1.5 to 4.5 mM in healthy people. The quantification of UA in urine and blood samples is of significant clinical importance as it can assist in diagnosing organism dysfunctions. The most commonly used methods for determining UA in biological samples are chromatographic, voltammetric, and capillary electrophoresis (TROIANI, 2011).

Nanomaterials have received significant attention in recent years due to their great potential for application in diverse fields such as chemistry, biochemistry, electronics, mechanics, and optics. An essential group within these nanoscale materials consists of carbon nanotubes (CNT), discovered in 1991 by Iijima. Since their discovery, CNTs have been the subject of numerous investigations, which is due to their structural, mechanical, and electronic properties, which make these materials very attractive nanostructures for a wide range of applications (Iijima et al., 2001; Ajayan, 1999).

Carbon nanotubes are formed from hexagonal arrangements of carbon that form small cylinders. They can be classified by the number of walls/layers, lengths, diameters, and the presence of functional groups, which can alter the interaction between cells or tissues with carbon nanotubes (Newman et al., 2013). Structurally, there are two types: single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNTs) (Ferreira, Rangel, 2009). A superficial layer of graphite represents SWCNTs rolled into a cylinder. This layer comprises carbon atoms forming a hexagonal network, with single and

double bonds and distances of 0.14 nm between nearby atoms. MWCNTs comprise two simple layers or more of coaxial cylinders (coinciding axes), closed at the ends with fullerene "hemispheres." They may present defects such as the presence of isolated pentagons and heptagons, and have a separation distance between layers of around 0.34 nm (Lobo et al., 2008).

MWCNTs are produced at low cost, and compared to most commercially available sensors based on metal oxides, silicon, and other materials, sensors based on carbon nanotubes (CNT) have the following advantages: high electrical conductivity and consequently decreased resistance to charge transfer; excellent chemical stability; broad application potential as molecular electronic components; the possibility of functionalization due to the presence of carboxylic groups; increase in surface area; high sensitivity, because of the large surface area, CNT can be used to immobilize enzymes, to maintain high biological activity; fast response time, have a remarkable ability to mediate electron transfer kinetics quickly; lower redox reaction potential and less surface fouling effects and high stability and lifetime (Pinho Júnior, 2016).

To this end, several techniques have been used to detect DA, AA, and UA, such as fluorimetry, chemiluminescence, ion exchange chromatography, high-performance liquid chromatography, ultraviolet-visible spectroscopy, and capillary electrophoresis. These techniques may present some disadvantages or inconveniences, as they are often complicated and often require pre-treatment of the sample, long analysis time, or a large number of organic solvents, which, in addition to being expensive, lead to the generation of a large amount of toxic waste. In this sense, electrochemical methods have received great attention due to their various advantages, such as low cost, high sensitivity and selectivity, speed of analysis, and ease of operation. Furthermore, these methods can be miniaturized and automated, enabling the construction of portable devices that allow rapid and efficient analysis of substances *in situ* (Oliveira, 2012).

The main goal of this work was to determine the concentration of ascorbic acid, uric acid, and dopamine on an unmodified carbon nanotube paste electrodes (CNTPE).

2. MATERIAL AND METHODS:

2.1. Materials

Potassium ferricyanide $K_4[Fe(CN)_6]$, NaOH, KNO_3, dibasic sodium phosphate, monobasic potassium phosphate, ascorbic acid, and H_3PO_4 purchased from Vetec®; HNO_3, dopamine (DA), Pipes, multiwalled carbon nanotubes, and mineral oil (Nujol) purchased from Sigma-Aldrich®, all reagents were grade P.A.

Phosphate buffers 0.10 M (pH = 7.0) and 50 mM (pH = 4.0), Pipes buffer 0.1 M (pH = 6.98), KCl 0.1 M, $K_4[Fe(CN)_6]$ 1.0 mM, stock solutions of ascorbic acid (AA) 200 ppm, 1762 ppm and 10 mM (phosphate buffer 50 mM), dopamine (DA) 200 ppm, and 0.010 M (phosphate buffer 0.050 M), uric acid (UA) 1.0 mM (NaOH 0.1 M) and NaOH 2.0 M. All solutions were prepared with deionized water and pH were measured in a pHmeter (Metrohm).

2.2. Methods

All voltammetric measurements were performed on a μAutolab potentiostat/galvanostat (Metrohm) connected to an electrochemical cell (25.0 mL) of three electrodes: working, reference (Ag/AgCl, $KCl_{3.0M}$) and auxiliary (platinum). Voltammetric measurements were recorded with the NOVA 2.1.5 software. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used for the electroanalytical study. Before each test, the solutions were always purged with N_2 gas for 5 minutes.

The working electrode was handmade in our laboratory by introducing a brass wire into a Teflon® cylinder, leaving a small space for placing the electrode material paste: carbon nanotubes (CNT) + mineral oil. A preliminary study optimized the voltammetric working conditions: better buffer, CNT + oil ratio, and sweeping speed. The following buffers were used: phosphate 0.10 M (pH = 6.98) and Pipes 0.10 M (pH = 6.90).

As shown in Table 1, there is no significant difference between the currents measured in each buffer. However, the phosphate buffer was chosen because several authors (Yao et al., 2007; Lin et al., 2008) claim to be the most used and appropriate for this study.

Another optimized condition was the proportion of CNT and mineral oil for paste formation. The proportion chosen was 50%CNT: 50%mineral oil (m/m), as in this case, the paste had better adhesion and consistency than the proportion 65%CNT: 35%mineral oil (m/m).

Regarding the scanning speed, due to the oxidation of DA presenting a slow electron transfer kinetics, the voltammetric recordings were carried out at 10 $mV{\cdot}s^{-1}$. The following proportions were used to prepare the paste: 50% CNT + 50% mineral oil and 65% CNT + 35% mineral oil.

Before using CNTPE, it is necessary to estimate the effective surface area of this electrode, as the surface area occupied by carbon nanotubes is unknown. The area was estimated from data obtained by cyclic voltammetry for the reversible pair $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ and Randles-Sevcik equation (Brett & Brett, 1996). The effective area (*A*) of CNTPE was estimated to be 0.084 cm^2.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Study of the voltammetric behavior of DA, AA, and UA on carbon nanotube paste electrode

In the study of electrochemical behavior, 50 mM phosphate buffer (pH = 4.0) was used as a supporting electrolyte; according to Yao et al. (2007), under these conditions, there is an improvement in the electrocatalytic activity for the oxidations of dopamine (DA), uric acid (UA) and ascorbic acid (AA), facilitating the lowering of the excess potential of DA. Initially, this study was carried out using cyclic voltammetry. A mixture of these three analytes with concentrations: AA 70 µM; UA 10 µM and DA 20 µM were used to observe each species' individual behavior.

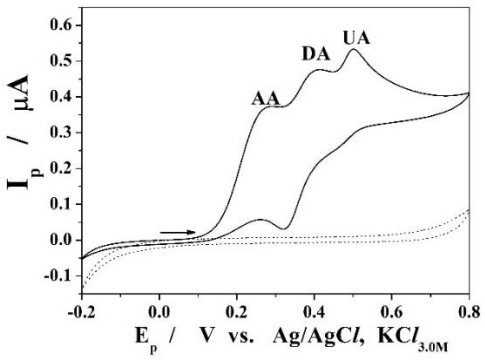

Figure 4. Cyclic voltammograms were recorded on CNTPE in 50 mM phosphate buffer (pH = 4.0) and mixture: 70 µM AA + UA 10 µM + DA 20 µM. Experimental: $E_i = E_f = 0.0$ V; $E_{\lambda1} = -0.20$ V; $E_{\lambda2} = 0.80$ V, and $\nu = 10$ $mV.s^{-1}$. Source: the authors.

It's possible to observe in Figure 4 the presence of all perfectly separated oxidation and reduction peaks. However, they present low currents. Therefore, this CNTPE, without chemical modification, could reproduce the records of modified electrode surfaces described in the literature (Yao et al., 2007; Lin et al., 2008).

As can be seen in Figure 5, three AA, DA, and UA oxidation peaks can be observed at different potentials of 200 mV, 380 mV, and 470 mV, respectively, with a potential difference between AA and DA of 180 mV and 90 mV between DA and UA. Since DPV has higher current sensitivity and better resolution than CV, the DPV was used for the electroanalytical determination of DA, AA, and UA on the CNTPE.

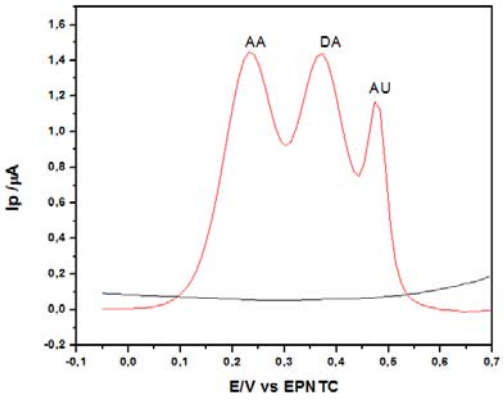

Figure 5. Differential pulse voltammograms were recorded over CNTPE in 50 mM phosphate buffer (pH = 4.0) containing 70 µM AA, 10 µM UA, and 20 µM DA. Experimental: $\nu = 5.0$ $mV{\cdot}s^{-1}$, $A_p = 20$ mV, and $\Delta E_i = 10$ mV. Source: the authors.

3.1.2. Determination of AA, UA, and DA in natural water by DPV

Three separate recordings were made once the experimental parameters for using DPV were defined. For each one, the concentration of one of the analytes was increased with successive additions of the solution of one of the analytes. In contrast, the concentrations of the other two species were maintained constants. The results are shown in Figure 6.

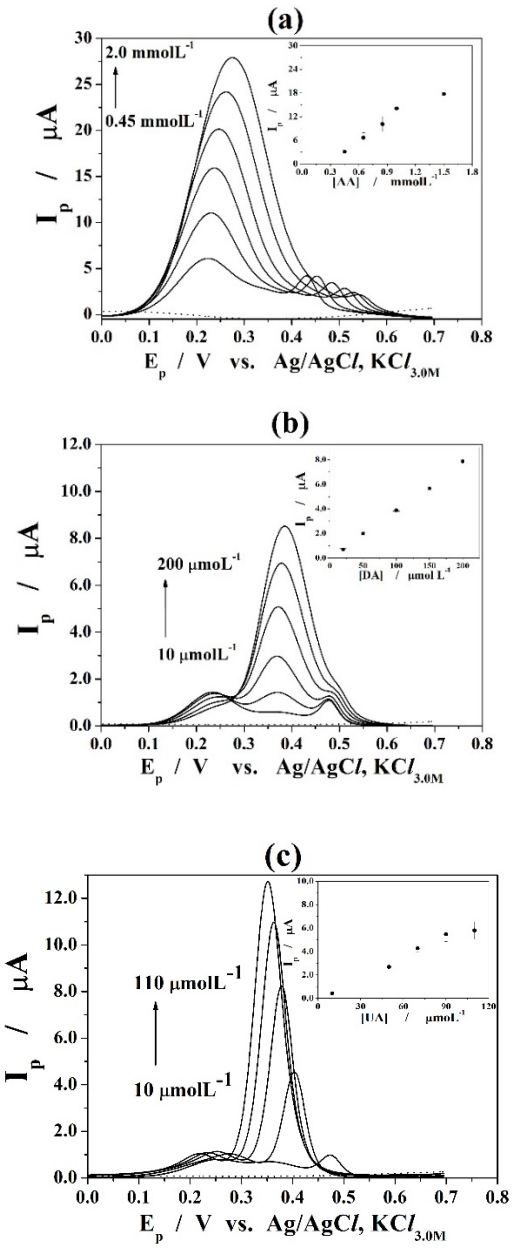

Figure 6. Differential pulse voltammograms were recorded over CNTPE in 50 mM phosphate buffer (pH = 4.0) with increasing additions of AA for 50 μM DA + 50 μM UA (a), DA for 10 μM UA + 70 μmol·L^{-1} AA (b), and UA for 40 μM AA + 10 μM DA (c). Experimental: ν = 5.0 mV s^{-1}, A_p = 20 mV and ΔE_i = 10 mV. Equations of the linear lines inserted in the figure, 6(a): I_p (μA) = –5.74 + 20.3 (± 0.8) μM [AA], R^2 = 0.9953; 6(b): I_p (μA) = –0.062 + 39.2 (± 0.00) *n*M [DA], R^2 = 0.9986; 6(c): I_p (μA) = -0.159 + 58.8 (± 0.00) *n*M [UA], R^2 = 0.9947. Source: the authors.

The linear ranges for the simultaneous determination of AA, DA, and UA by DPV were 0.45 – 1.0 mM, 50 – 200 μM and 10 – 90 μM, respectively (Figure 6). The limits of detection (LODs) of the proposed method for determining AA, DA, and UA were 7.97 mM, 8.57 μM and 5.96 μM, respectively. The relative standard deviation of 10 successive scans was 4.6, 2.8, and 1.6% for 0.45 mM of AA, 50 μM of DA, and 50 μM of UA.

3.2. Discussion

3.2.1. Study of the voltammetric behavior of DA, AA, and UA on carbon nanotube paste electrode

Figure 4 presents peaks corresponding to AA and UA have potentials of 225 mV and 500 mV, respectively. The peak corresponding to DA presented a redox couple, which commonly only appears in this analyte (Lin et al., 2008).

The anodic and cathodic peak potentials for DA are 400 mV and 350 mV, respectively. The oxidation mechanism of AA, UA, and DA is a process that involves two electrons and 2 protons, and it agrees with the mechanism presented by Zhao et al. (2005). According to Lin et al. (2008), electrochemical detection of DA in the presence of high levels of AA on carbon-based electrodes becomes difficult due to the catalytic oxidation of AA by DA. But, as shown in Figure 5, the CNTPE achieved a good voltammetric response in the ternary mixture of these analytes.

3.2.2. Determination of AA, UA, and DA in water natural water by DPV

During the additions of the standard to the ternary mixture (Fig. 6), it was observed that the potentials of AA and DA were shifted to more positive values, while UA behaved differently, as the potential was shifted to more negative values. It is unknown whether this new electrode surface causes this behavior or is due to another event, as this behavior has not yet been reported in the literature.

For the three molecules, AA, UA, and DA, it is observed that the oxidation peak currents increased with increasing concentration. No change in DA and UA currents was observed after increasing the concentration of AA; however, during the increasing addition of UA, it was observed that its potential was shifted to more negative values, leading UA to approach the oxidation potential of DA, causing possible interference in the quantitative determination of UA. In this case, UA needs to improve the analysis conditions.

4. CONCLUSIONS:

This work presents the advantages of CNTPE without chemical modification for the simultaneous determination of species of biological interest: ascorbic acid (AA), uric acid (UA), and dopamine (DA), as it showed good electrocatalytic activity for the oxidation of these species. Due to the chemical stability of carbon nanotubes, this electrode surface can be used in electroanalysis as an electron transfer mediator. Optimizing the voltammetric working conditions indicated that the electrode's electrochemical behavior strongly depends on the pH of the solution. The CNTPE allowed the separation of the oxidation peaks of the ternary mixture of AA, UA, and DA by cyclic voltammetry. The limits of detection (LODs) of the proposed method for determining AA, DA, and UA were 7.97 mM, 8.57 μM, and 5.96 μM, respectively. Therefore, it showed quantitative application when associated with differentiated pulse voltammetry, as it allows the simultaneous determination of these biological compounds in an aqueous solution with good sensitivity and selectivity.

5. DECLARATIONS

5.1. Study limitations

AA, UA, and DA determination studies were also carried out using two other electrode surfaces made of carbon: glassy carbon (GCE) and graphite paste (CPE).

The previously assembled CPE and CNTPE had a limited useful life, possibly due to the support electrolytes (buffers B-R) used, as the higher the pH of the buffer, the greater the attack suffered by these electrode surfaces and consequently the shorter the useful life of these electrodes. However, this disadvantage is fully compensated due to the renewal of the electrode surface, when “poisoning” of the electrode is observed.

The choice to use CNTPE and CPE is justified for the following reasons: (i) greater hydrophobicity of these electrodes compared to the glassy carbon electrode (GCE), due to the presence of Nujol oil, hoping to have more success in stabilizing the analytes (AA, UA and DA) in aqueous medium, (ii) lower background currents and (iii) ease of renewal of the carbon nanotube paste, thus avoiding undesirable adsorption effects and time-consuming GCE polishing steps. In comparison to CPE, the choice for CNTPE was due to the better resolution of voltammetric recordings, and this is associated with the intrinsic properties of carbon nanotubes: wide working potential window, low background current, and eventual stabilization of ionic species and/or molecular.

The data presented in Table 1 indicate the reason for choosing phosphate buffer as the supporting electrolyte.

As this is preliminary work and we have not yet submitted the project to the Ethics Committee, it was not possible to extend the research to biological samples (data not shown).

5.2. Acknowledgements

To other research groups at UVA/CE.

5.3. Funding source

To funding agencies: CNPq for granting research grants. Hélcio Silva dos Santos and Murilo Sérgio da Silva Julião are grateful for the financial support provided by PQ/CNPq (Grant#: 306008/2022-0) and FUNCAP (Grant#: BP5-0197-00087.01.00122), respectively.

5.4. Interest conflicts

We declare that we do not have any potential conflict of interest in this publication.

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Table 1. *Optimization of working conditions: proportion of CNT + mineral oil; buffer and scan rate (ν). Source: the authors.*

	Scan rate (V s^{-1})			
	0.01	**0.05**	**0.01**	**0,05**
Analyte	**CNT 50% + mineral oil 50%**		**CNT 65% + mineral oil 35%**	
Pipes 0.10 M buffer (pH = 6.90).				
AA	3.22 µA	7.10 µA	5.37 µA	7.25 µA
UA	8.00 µA	9.23 µA	7.08 µA	8.27 µA
DA	2.62 µA	1.59 µA	4.73 µA	9.12 µA
AA, UA, DA	3.03 µA	0.413 µA	1.07 µA	2.19 µA
Phosphate 0.10 M buffer (pH = 6.98)				
AA	7.10 µA	9.22 µA	1.07 µA	1.56 µA
UA	5.86 µA	8.58 µA	11.4 µA	14.4 µA
DA	2.10 µA	1.71 µA	9.43 µA	17.1 µA
AA, UA, DA	3.19 µA	3.42 µA	3.15 µA	1.62 µA