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Towards Detection of Total Antioxidant Concentrations of Glutathione, Cysteine, Homocysteine and Ascorbic Acid Using a Nanocarbon Paste Electrode

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The electrocatalytic reaction between catechol and the antioxidants, glutathione, cysteine, homocysteine and ascorbic acid is studied at a nanocarbon paste electrode and used to measure the *total* antioxidant concentration in aqueous solution. Two different approaches are described: one in which catechol is dissolved in solution and the second in which catechol is dissolved into the nanocarbon paste electrode. Similar limits of detection of 2.0μM and 1.9μM and sensitivities of $8.8x10^{-3}$μA/μM and 0.11$μM^{-1}$ are reported, respectively at nanocarbon and nanocarbon-catechol paste electrodes. Three different commercial multivitamin drug samples were analysed and the results were in a good agreement with those from independent analysis.

Keywords: Nanocarbon paste electrode, antioxidants, catechol, glutathione, cysteine, homocysteine, ascorbic acid

1. INTRODUCTION

Free radicals are reactive molecules arising physiologically during cellular aerobic metabolism. Their possible harmful effects for human health have raised increasing concern in recent years [1]. They react rapidly with other compounds sometimes causing chain reactions. The resulting biochemical alterations are implicated in a growing list of human diseases, such as cardiovascular diseases, ageing, Parkinson's disease, Alzheimer's disease, diabetes and cancer [2, 3]. Antioxidants are

compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions [4]. Physiologically the most relevant antioxidants include tocopherol (vitamin E), retinol (vitamin A), ascorbic acid (vitamin C), glutathione, cysteine and uric acid [3, 5, 6].

Most studies in the literature for the electrochemical detection of antioxidants are focused on the food industry [1, 7, 8] whereas in the biological area relatively only a few articles can be found [9, 10]. The purpose of this paper is to present a method for the electrocatalytic detection of the total biological concentrations of glutathione, cysteine, homocysteine and ascorbic acid using a nanocarbon paste electrode via reaction between an electrochemically generated *ortho*-quinone and the antioxidants. These four antioxidants are chosen because they are found at high concentration in biological samples.

Glutathione is the most prevalent cellular thiol and the most abundant low-molecular-weight peptide present in cells. Glutathione acts an antioxidant, participating in detoxification for xenobiotics and metabolism of numerous cellular compounds [11]. Homocysteine is an endogenous sulfhydryl amino acid, which is generated by the demethylation of methionine and your level in plasma or serum is a sensitive indicator of vitamin B_{12} and folate deficiencies [12]. Cysteine is sulfur containing amino acid and known to play an important role in various biochemical processes [13]. As an antioxidant, ascorbic acid scavenges free radicals in the body and protects tissues from oxidative stress. Ascorbic acid also promotes the adsorption of iron, while preventing its oxidation [6].

Nanocarbon has recently been proposed as useful electrode material which offers similar advantages to other carbon electrodes but at close to zero cost [14, 15]. Lo et al. [14] reported the advantages of using nanocarbon as an alternative electrode modifier for use in adsorptive stripping voltammetry. In this case, the nanocarbon particles were cast onto a glassy carbon electrode. Lowinsohn et al. [15] investigated the electrochemical behaviour of carbon paste electrodes prepared using nanocarbon and mineral oil. In this case, the nanocarbon particles were mixed with mineral oil producing the paste.

Two possible reactions can occur between an *o*-quinone group and a thiol containing molecule: 1,4-Michael addition and/or electrocatalytic reaction [16-21]. These are illustrated in Figure 1 where oxidation of catechol is used to form *o*-quinone (leading to a current $I_{catechol}$). As a result of 1,4-Michael addition, the voltammogram (Figure 1A) can show the introduction of a new signal (P1) and at maximum, with excess thiol, a four-electron process is seen for the catechol oxidation signal ($I_{catechol+thiol} \leq 2I_{catechol}$). During the electrocatalytic reaction, the catechol undergoes an electrochemical oxidation generating *o*-quinone which can mediate the oxidation of the thiol to produce disulfide [16-21]. Subsequently, the catechol can be electrochemically re-oxidized. For this mechanism, the oxidation peak will increase and the reduction peak will decrease (Figure 1B) as the concentration of the thiol species increases and no new signal will appear in the voltammograms as the reaction is exclusively based on the quinone/hydroquinone redox reaction. For fast reactions and large concentrations, the catechol signal can show an effective number of electrons transferred in excess of two or even four. In this paper, we explore the electrocatalytic reaction in order to detect glutathione, homocysteine, cysteine and ascorbic acid as total antioxidants.

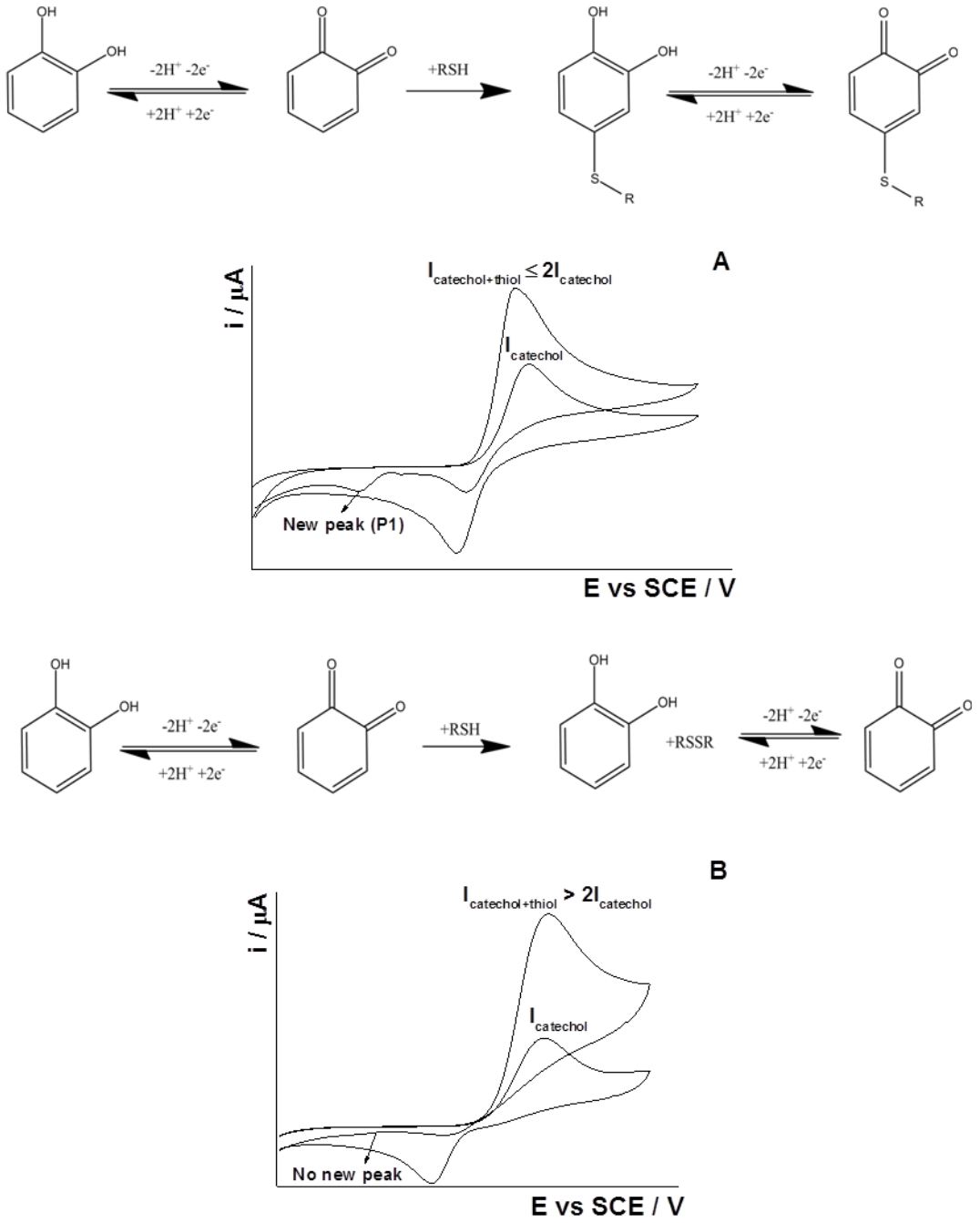


Figure 1. Resulting voltammograms of a solution containing catechol ($I_{catechol}$) and thiol ($I_{catechol+thiol}$) via: (A) 1,4 –Michael addition and (B) Electrocatalytic reaction.

2 EXPERIMENTAL SECTION

2.1 Chemicals

Catechol ($C_6H_6O_2$, Aldrich), L-cysteine ($C_3H_7NO_2S$, Sigma-Aldrich), DL-homocysteine ($C_4H_9NO_2S$, Sigma-Aldrich), glutathione ($C_{10}H_{17}N_3O_6S$, Sigma-Aldrich), ascorbic acid ($C_6H_8O_6$, Sigma-Aldrich), potassium phosphate dibasic (K_2HPO_4, Aldrich), potassium phosphate monobasic

(KH_2PO_4, Sigma), nanocarbon particles (diameter 27 ± 10 nm, Monarch 430®, Cabot Performance), mineral oil (Aldrich) were used as received without further purification. Phosphate buffer solution (PBS) was prepared using the adequate amount of K_2HPO_4 and KH_2PO_4 salts. All solutions were prepared using deionised water of resistivity not less than 18.2 MΩ cm at 25°C (Millipore, Billerica, MA, USA). Prior to experiments, all solutions were purged through nitrogen (N_2, BOC, Surrey) to remove oxygen from the system.

2.2 Instrumental

All electrochemical experiments were conducted at (25±1)°C using a Autolab (Eco Chimie,Utrecht, The Netherlands), with a standard three-electrode configuration consisting of nanocarbon or nanocarbon-catechol paste as a working electrode, a graphite rod as a counter electrode and a saturated calomel electrode (SCE) as reference electrode. All experiments were performed at least three times.

2.3 Preparation of nanocarbon and nanocarbon-catechol paste electrode

Nanocarbon: The carbon paste was prepared by hand pasting nanocarbon with mineral oil (55:45) using a pestle and mortar [15]. The pastes were kept at room temperature until used.

Nanocarbon-catechol: Catechol solution was prepared with a certain amount of solid catechol dissolved in acetone. Catechol is not soluble in mineral oil so the acetone is employed to initially dissolve it. Modified nanocarbon-catechol paste electrode was prepared by hand pasting nanocarbon with mineral oil and an aliquot of catechol solution using a pestle and mortar. The resulting paste was left for at least 30 min. in nitrogen atmosphere to evaporate the acetone. After the evaporation, solid catechol is assumed to be distributed within in the paste. The pastes were kept in nitrogen atmosphere at room temperature until used to avoid catechol oxidation.

For both pastes, unmodified and catechol-modified, the material was packed into the well of the working electrode to a depth of 1 mm. The surface exposed to the solution was polished using a weighing paper to give a smooth finish before use. The body of the working electrode was a Teflon tube tightly packed with the carbon paste. The electrical contact was provided by a copper wire.

2.4 Samples

Antioxidants content of multivitamin drugs was determined by using the proposed method at unmodified and modified nanocarbon paste electrode. Five tables were weighed and triturated. The sample stock solutions were prepared by dissolving approximately 20mg of powder in PBS in a 10mL volumetric flask. Working solutions were prepared by the dilution of suitable volumes from the stock. Sample 1 contains ascorbic acid, cysteine and glutathione. Samples 2 and 3 contain ascorbic acid and glutathione.

3. RESULTS AND DISCUSSION

3.1 Electrochemical behaviour of catechol in a nanocarbon paste electrode

Initially, voltammetric studies at different scan rates (from 50 to 800mVs^{-1}) involving the electrochemical behaviour of catechol in phosphate buffer solution (pH = 7.5) using the nanocarbon paste electrode were performed (Figure 2). The voltammograms show a chemically reversible redox process with a formal potential at ca +0.17V (vs SCE).

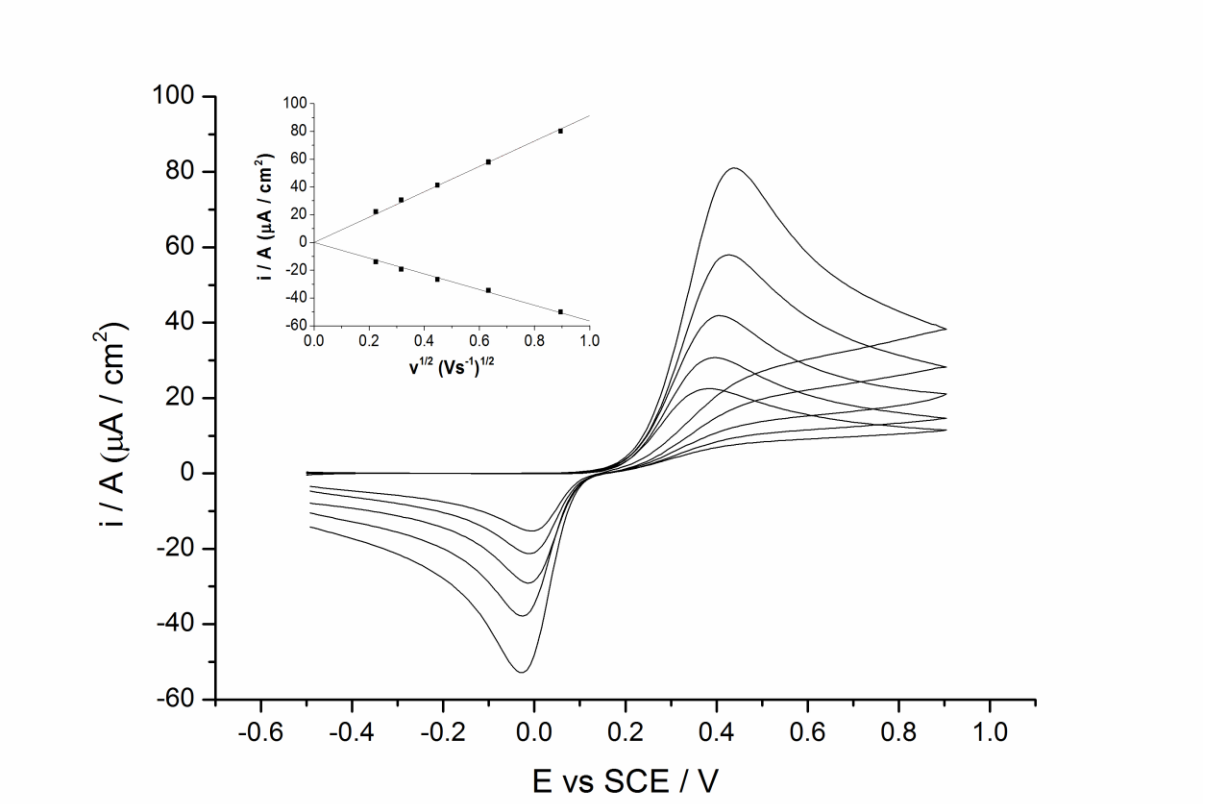
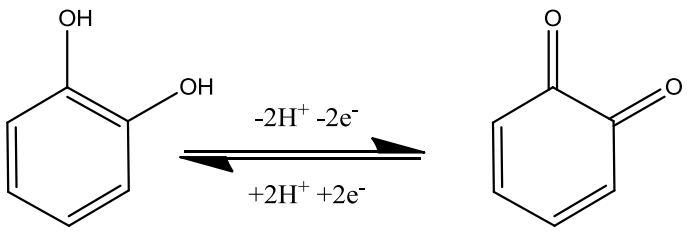


Figure 2. Cyclic voltammetric responses for 100μM catechol in PBS (pH = 7.5) at different scan rate (from 50 to 800 mVs^{-1}) at nanocarbon electrode. Inset: plot of peak current against square root of scan rate. The current was normalized by electrode area. E^0 = +0.17V and D = 7.0x10^{-6} cm^2/s

This process is attributed to the two electron oxidation of catechol to the corresponding *o*-quinone species:



This value is close to that reported in the literature [22]. The inset in Figure 2 shows the peak current increased linearly with the square root of scan rate, suggesting a diffusional process of catechol

at this electrode. The catechol diffusion coefficient value was estimated as being $7.0x10^{-6}cm^2/s$. This is reasonably consistent with the value found in the literature ($7.7x10^{-6}$ cm^2/s) [23].

3.2 Catechol in solution: detection of the antioxidants - glutathione, cysteine, homocysteine and ascorbic acid

Next, the characterization of the reaction between the electrochemically generated *o*-quinone and the antioxidants, glutathione, cysteine, homocysteine and ascorbic acid, was carried out at a nanocarbon paste electrode using cyclic voltammetry ($100mVs^{-1}$). The corresponding voltammetric responses of 100µM catechol (pH = 7.5) in the absence (curve a) and presence (curves b and c) is of glutathione (A), homocysteine (B), cysteine (C) and ascorbic acid (D) are detailed in Figure 3.

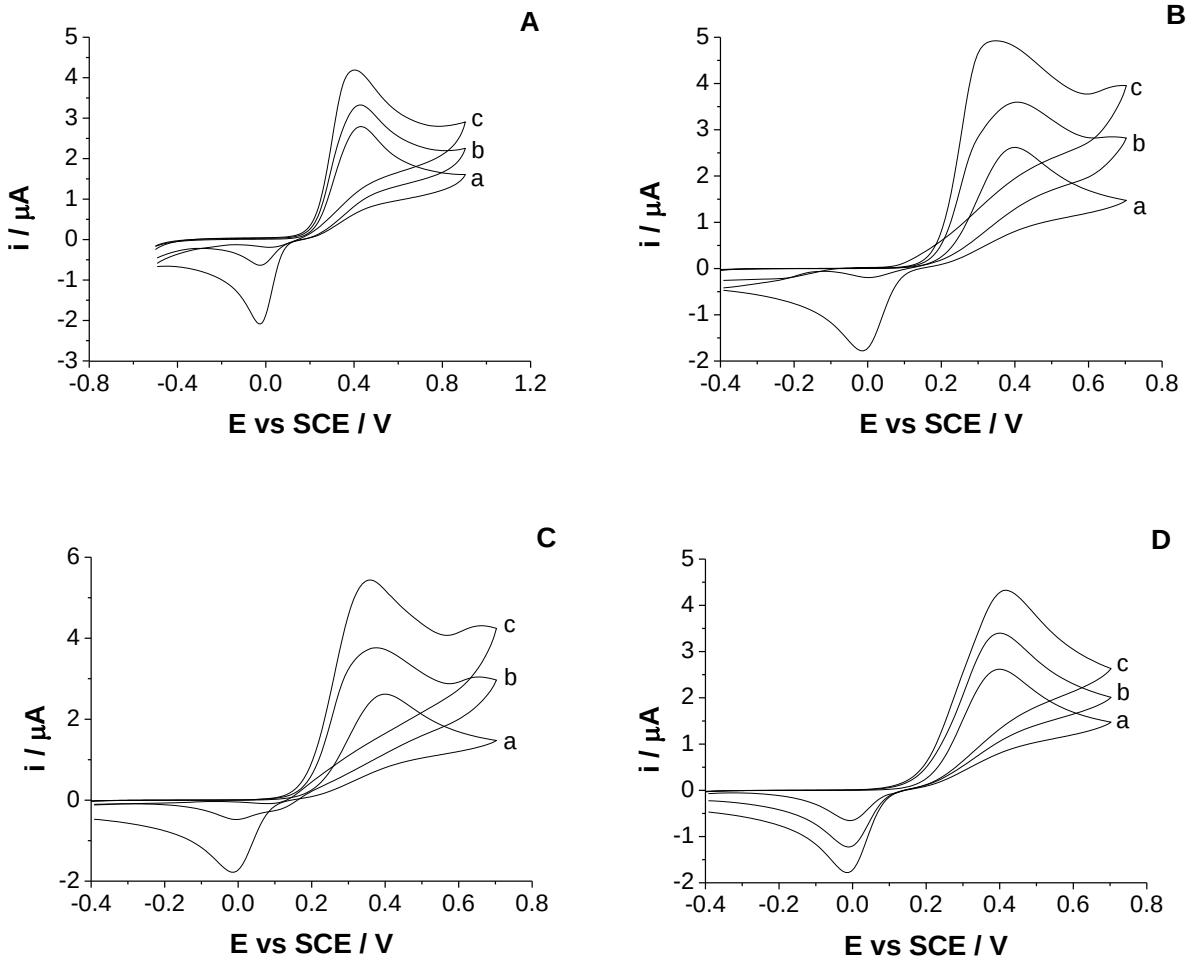


Figure 3. Cyclic voltammetric responses for 100µM catechol in PBS (pH = 7.5) in the absence (a) and presence of glutathione (A), homocysteine (B), cysteine (C) and ascorbic acid (D) using nanocarbon paste electrode. Different concentrations were added (b) 40 and (c) 100µM. v = $100mVs^{-1}$.

In all cases, an analytical curve was obtained by plotting oxidation peak current (I_o) versus concentration (Figure 4). Note that in some situations it was not possible to measure the reduction

current peak, because at high concentration of the analytes, it decreases to zero. The sensitivity for cysteine is 0.026μA/μM, for glutathione is 0.014μA/μM, for homocysteine is 0.023μA/μM and for ascorbic acid is 0.016μA/μM. All these sensitivities were obtained in pure solution. The sensitivities differ a little from compound to compound. The data show that the system is more sensitive to cysteine than glutathione, probably owing to the small size of the molecule leading to enhanced reactivity. Homocysteine and cysteine show similar sensitivity. The detection limits for each individual compound are: 1.7, 3.2, 2.0 and 2.8μM, respectively, for cysteine, glutathione, homocysteine and ascorbic acid.

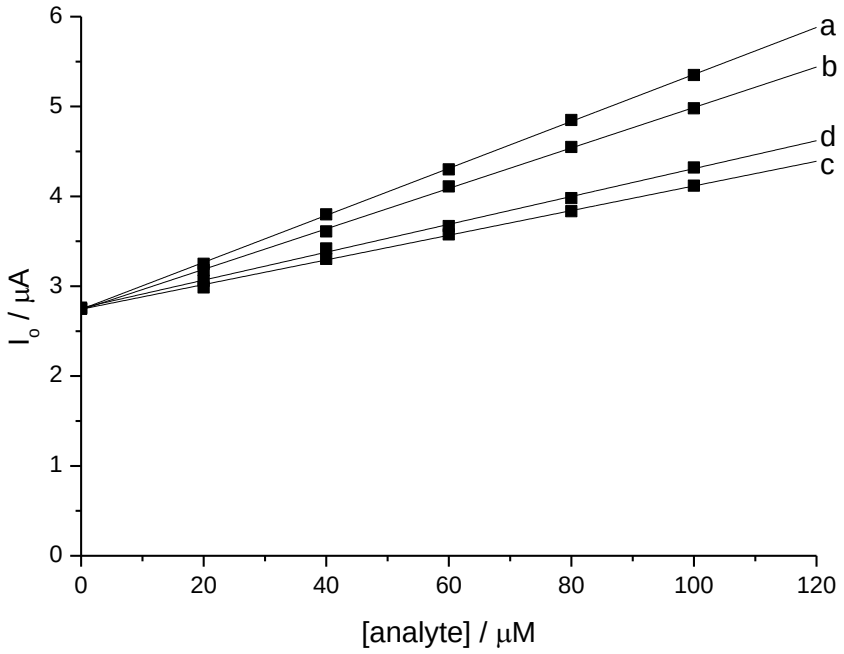


Figure 4. Calibration curve for (a) cysteine, (b) homocysteine, (c) glutathione and (d) ascorbic acid using a nanocarbon paste electrode and catechol in solution. [Catechol] = 100μM.

The nanocarbon paste electrode is not selective; all the analytes showed similar responses. However this observation enables us to propose a strategy for the detection of the *total* concentrations. Accordingly, cyclic voltammograms were carried out in mixtures of glutathione, homocysteine, cysteine and ascorbic acid. Different total concentrations were chosen from 5 to 100μM. For each one, different mixtures were prepared as shown in Table 1.

Figure 5 shows the cyclic voltammograms response for different total antioxidants mixtures and the analytical curve (inset). The linear equation obtained was I_o (μA) = 2.409 + 0.0088[antioxidants] (μM). The reproducibility for each different total concentration was better than 5% (n = 3) and the detection limit was 2.0μM, demonstrating that the nanocarbon paste electrode is efficient for the detection of total antioxidant concentrations.

Table 1. Different mixtures and the oxidation current (I_o) obtained from cyclic voltammetry using nanocarbon paste electrode.

Mix	[Glutathione] / μM	[Homocysteine] / μM	[Cysteine] / μM	[Ascorbic acid] / μM	[Total] / μM	I_o / μA
A	25.0	25.0	25.0	25.0	100	3.41
B	25.0	25.0	35.0	15.0	100	3.29
C	25.0	25.0	15.0	35.0	100	3.16
D	25.0	35.0	15.0	25.0	100	3.32
E	25.0	15.0	35.0	25.0	100	3.10
F	35.0	15.0	25.0	25.0	100	3.28
G	15.0	35.0	25.0	25.0	100	3.39
H	15.0	25.0	25.0	35.0	100	3.49
I	35.0	25.0	25.0	15.0	100	3.32
A	20.0	20.0	20.0	20.0	80.0	2.88
B	20.0	20.0	30.0	10.0	80.0	3.04
C	20.0	20.0	10.0	30.0	80.0	3.12
D	20.0	30.0	10.0	20.0	80.0	3.17
E	20.0	10.0	30.0	20.0	80.0	3.14
F	30.0	10.0	20.0	20.0	80.0	3.13
G	10.0	30.0	20.0	20.0	80.0	3.19
H	10.0	20.0	20.0	30.0	80.0	3.05
I	30.0	20.0	20.0	10.0	80.0	3.26
A	10.0	10.0	10.0	10.0	40.0	2.66
B	10.0	10.0	15.0	5.00	40.0	3.04
C	10.0	10.0	5.00	15.0	40.0	2.76
D	10.0	15.0	5.00	10.0	40.0	2.73
E	10.0	5.00	15.0	10.0	40.0	2.83
F	15.0	5.00	10.0	10.0	40.0	2.71
G	5.00	15.0	10.0	10.0	40.0	2.72
H	5.00	10.0	10.0	15.0	40.0	2.76
I	15.0	10.0	10.0	5.00	40.0	2.71
A	5.00	5.00	5.00	5.00	20.0	2.64
B	5.00	5.00	7.50	2.50	20.0	2.57
C	5.00	5.00	2.50	7.50	20.0	2.57
D	5.00	7.50	2.50	5.00	20.0	2.55
E	5.00	2.50	7.50	5.00	20.0	2.58
F	7.50	2.50	5.00	5.00	20.0	2.52
G	2.50	7.50	5.00	5.00	20.0	2.56
H	2.50	5.00	5.00	7.50	20.0	2.65
I	7.50	5.00	5.00	2.50	20.0	2.61
A	2.50	2.50	2.50	2.50	10.0	2.50
B	2.50	2.50	3.75	1.25	10.0	2.49
C	2.50	2.50	1.25	3.75	10.0	2.50
D	2.50	3.75	1.25	2.50	10.0	2.45
E	2.50	1.25	3.75	2.50	10.0	2.46
F	3.75	1.25	2.50	2.50	10.0	2.54
G	1.25	3.75	2.50	2.50	10.0	2.55
H	1.25	2.50	2.50	3.75	10.0	2.51
I	3.75	2.50	2.50	1.25	10.0	2.42
A	1.25	1.25	1.25	1.25	5.00	2.41
B	1.25	1.25	2.00	0.500	5.00	2.46
C	1.25	1.25	0.500	2.00	5.00	2.47
D	1.25	2.00	0.500	1.25	5.00	2.42
E	1.25	0.500	2.00	1.25	5.00	2.52
F	2.00	0.500	1.25	1.25	5.00	2.45
G	0.500	2.00	1.25	1.25	5.00	2.45
H	0.500	1.25	1.25	2.00	5.00	2.40
I	2.00	1.25	1.25	0.500	5.00	2.41

These values reflect the analytical results for the range of composition studied in Table 1: 10% < [analyte] < 40%, this in turn reflects the use in the medicine analysis reported below. Of course in the worst case scenario when 100% of glutathione or 100% of cysteine happen, the method would give a different range of values, reflecting the sensitivity values reported above. Using the equation obtained for total antioxidants (I_o (μA) = 2.409 + 0.0088[antioxidants] (μM)) to calculate the concentration of 100% glutathione (100μM - I_o = 4.11) or 100% cysteine (100μM - I_o = 5.35), values of 193μM and 334μM are, respectively, obtained. Both values are over estimates because the sensitivities of pure individual molecules (cysteine 0.026μA/μM, glutathione 0.014μA/μM) and total antioxidants (0.0088 μA/μM) are not similar as each molecule contributes differently in the absence or presence of other compounds. However in the application to biological samples or multivitamin drugs, the antioxidants are found mixed not isolated. In any case analyses to within a certain tolerance are valuable.

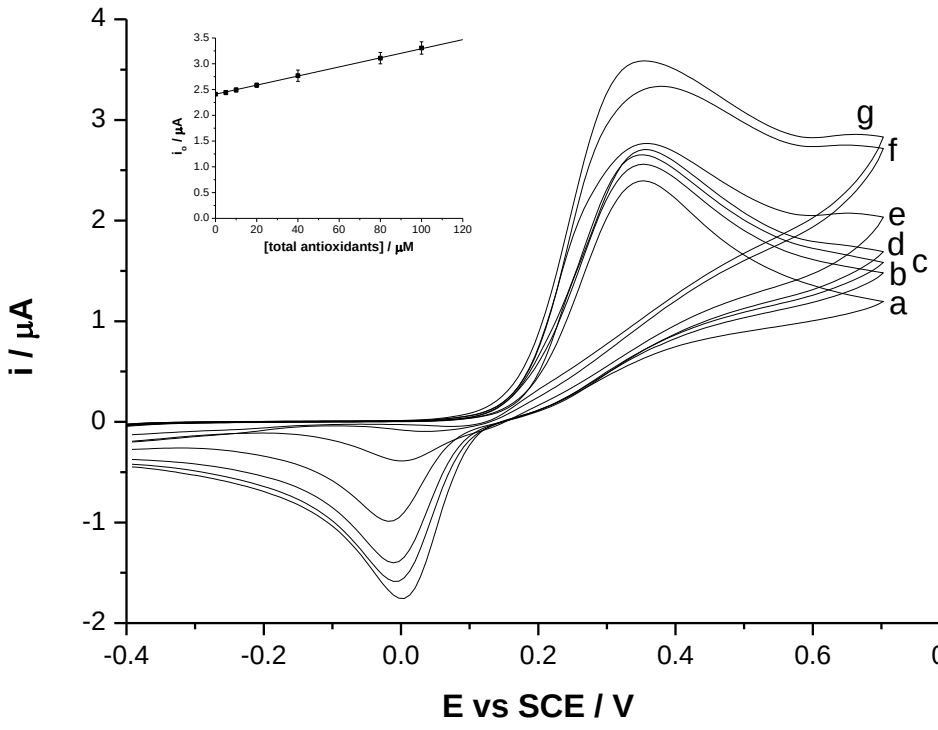


Figure 5. Cyclic voltammetric responses for 100μM catechol in PBS (pH = 7.5) in the absence (a) and presence of total antioxidants at different concentrations (b) 5μM, (c) 10μM, (d) 20μM, (e) 40μM, (f) 80μM and (g) 100μM using nanocarbon paste electrode. v = 100mVs^{-1}. Inset: analytical curve.

3.3 Catechol in nanocarbon paste: detection of antioxidants - glutathione, cysteine, homocysteine and ascorbic acid

A possible useful alternative to the method reported above is the use of catechol mixed with nanocarbon paste so as to create a reagentless sensor. In this case, the experiment requires only the

presence of the target to provide a signal and after polishing the paste and washed free of analyte, the sensor is ready for reuse.

Different percentages of catechol dissolved in the paste (0.03, 0.05, 0.1, 0.3, 0.6 and 1%) were used to make the modified electrode. Figure 6 shows cyclic voltammetric responses for 0.05 and 0.3% nanocarbon-catechol paste in PBS (pH = 7.5) at $100mVs^{-1}$. In both cases, similar electrochemical behaviour to catechol in solution was observed. Three scans with the same paste are presented in Figure 6, showing that the electrode surface area is not reproducible (standard deviation is about 53%); this is probably due to the low amount of catechol generating limited homogeneity in the paste. Because of this irreproducible signal from electrode to electrode the absolute height of the oxidation or reduction peak cannot be used (without standard additions). To solve this problem we decide to work with the *ratio* between the oxidation (I_o) and reduction (I_r) peak currents. The standard deviation in this case was 3.8% (n = 3).

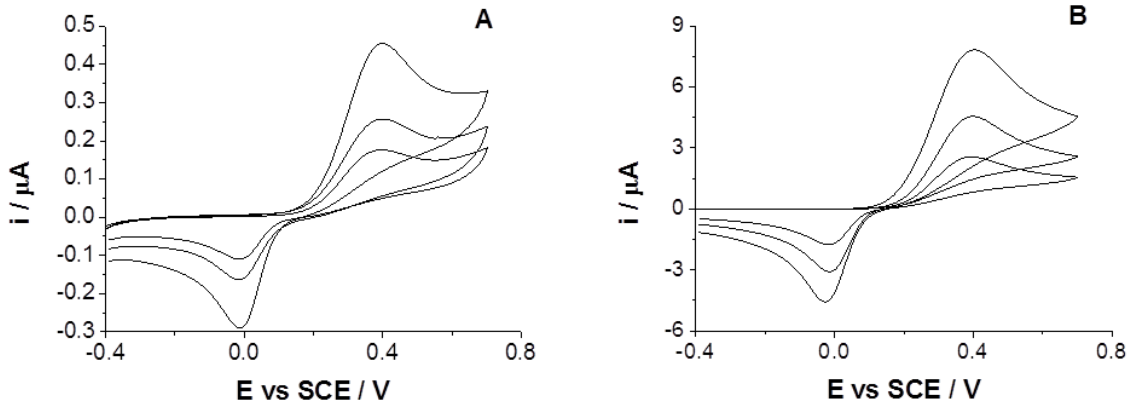


Figure 6. Cyclic voltammetric responses for 0.05% (A) and 0.3% (B) catechol dissolved in the nanocarbon paste in PBS (pH = 7.5) at $100mVs^{-1}$. Three scans with the same paste.

Using pastes with percentages of catechol 0.03, 0.05 and 0.1%, the sensor can quantify less than 20µM of antioxidants and using pastes with percentages of catechol 0.3, 0.6 and 1%, the electrode can detect up to 80µM of antioxidants with different sensitivity. Nanocarbon pastes with high percentages of catechol lose sensitivity because low concentrations of antioxidants do not affect significantly the signal of catechol. For further studies, the modified electrode containing 0.3% of catechol was chosen because this electrode is more sensitive to the antioxidants and can detect a large range (up to 80µM) of antioxidants.

Cyclic voltammograms were recorded at $100mVs^{-1}$, using the nanocarbon-catechol paste chosen before, in different concentrations of (a) cysteine, (b) homocysteine, (c) glutathione and (d) ascorbic acid separately in order to obtain individual analytical curves. The calibration curves (Figure 7) shows a linear relationship for all individual antioxidants. The sensitivity for cysteine is $0.108\mu M^{-1}$, for glutathione is $0.076\mu M^{-1}$, for homocysteine is $0.088\mu M^{-1}$ and for ascorbic acid is $0.066\mu M^{-1}$. The data show that the nanocarbon-catechol paste electrode is more sensitive to cysteine, having a similar

behaviour to the nanocarbon paste electrode. The detection limits for each individual analyte are: 1.9, 1.2, 2.7 and 2.3μM, respectively, for cysteine, glutathione, homocysteine and ascorbic acid.

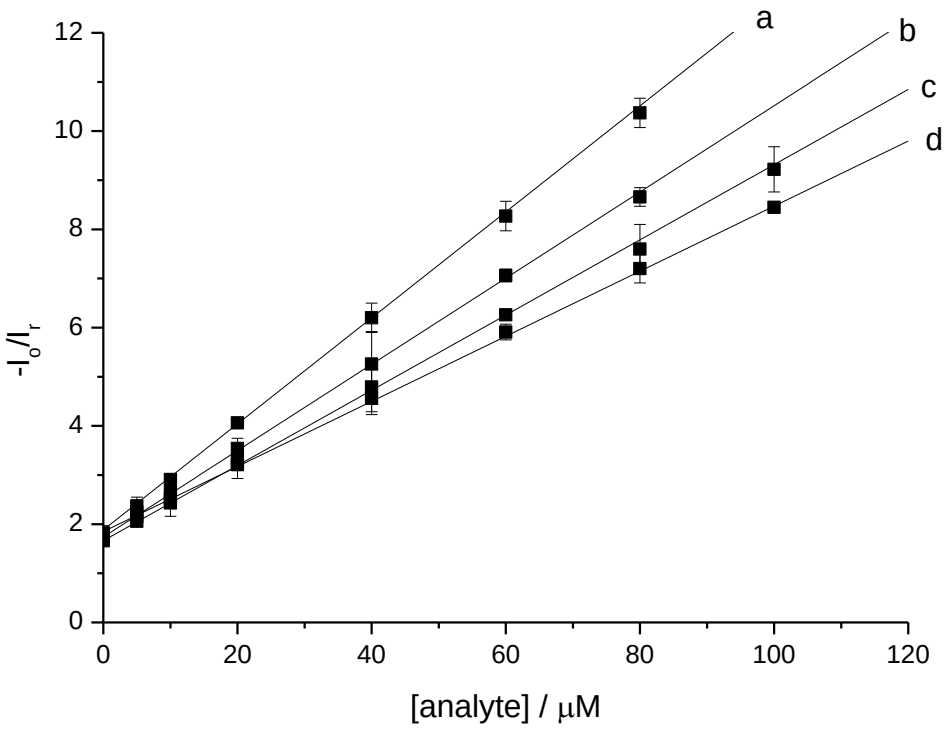


Figure 7. Calibration curve for (a) cysteine, (b) homocysteine, (c) glutathione and (d) ascorbic acid using nanocarbon-catechol paste electrode.

As the nanocarbon-catechol paste electrode is not totally selective various analytes which might interfere with the measurement of the antioxidants (urea, uric acid, creatinine, glucose and hydrogen peroxide) were tested and the results are shown in Table 2. The data show that the ratios obtained for all the tested analytes (creatinine: 1.80, glucose: 1.91, hydrogen peroxide: 1.76, urea: 1.86 and uric acid: 1.89) are very similar to that obtained only in pure PBS (1.85±0.07), indicating that these analytes do not react significantly with the *o*-quinone.

Table 2. Ratio between I_o and I_r for different analytes

Analytes	Concentration / μM	$-I_o/I_r$
Creatinine	100	(1.80±0.08)
Glucose	100	(1.91±0.08)
Hydrogen peroxide	100	(1.76±0.08)
Urea	100	(1.86±0.08)
Uric acid	100	(1.89±0.09)

*Nanocarbon-catechol paste in PBS: $-I_o/I_r$ = (1.85±0.07)

To detect the total concentration of glutathione, cysteine, homocysteine and ascorbic acid, cyclic voltammograms were carried out in various mixtures of glutathione, homocysteine, cysteine and ascorbic acid. Different total concentrations (glutathione, homocysteine, cysteine and ascorbic acid) were chosen from 5 to 80μM. For each one, different mixtures were prepared as shown in Table 3.

Table 3. Different mixtures and the ratio $-I_o/I_r$ (see text) obtained from cyclic voltammetry using nanocarbon-catechol paste electrode

Mix	[Glutathione] / μM	[Homocysteine] / μM	[Cysteine] / μM	[Ascorbic acid] / μM	[Total] / μM	$-I_o/I_r$
A	20.0	20.0	20.0	20.0	80.0	10.71
B	20.0	20.0	30.0	10.0	80.0	10.65
C	20.0	20.0	10.0	30.0	80.0	10.53
D	20.0	30.0	10.0	20.0	80.0	10.43
E	20.0	10.0	30.0	20.0	80.0	10.86
F	30.0	10.0	20.0	20.0	80.0	10.86
G	10.0	30.0	20.0	20.0	80.0	10.67
H	10.0	20.0	20.0	30.0	80.0	10.49
I	30.0	20.0	20.0	10.0	80.0	10.30
A	10.0	10.0	10.0	10.0	40.0	6.53
B	10.0	10.0	15.0	5.00	40.0	6.19
C	10.0	10.0	5.00	15.0	40.0	6.16
D	10.0	15.0	5.00	10.0	40.0	6.33
E	10.0	5.00	15.0	10.0	40.0	6.16
F	15.0	5.00	10.0	10.0	40.0	6.36
G	5.00	15.0	10.0	10.0	40.0	6.20
H	5.00	10.0	10.0	15.0	40.0	5.85
I	15.0	10.0	10.0	5.00	40.0	6.12
A	5.00	5.00	5.00	5.00	20.0	3.99
B	5.00	5.00	7.50	2.50	20.0	3.77
C	5.00	5.00	2.50	7.50	20.0	3.81
D	5.00	7.50	2.50	5.00	20.0	3.76
E	5.00	2.50	7.50	5.00	20.0	4.06
F	7.50	2.50	5.00	5.00	20.0	3.89
G	2.50	7.50	5.00	5.00	20.0	3.65
H	2.50	5.00	5.00	7.50	20.0	3.87
I	7.50	5.00	5.00	2.50	20.0	3.72
A	2.50	2.50	2.50	2.50	10.0	2.88
B	2.50	2.50	3.75	1.25	10.0	2.95
C	2.50	2.50	1.25	3.75	10.0	2.81
D	2.50	3.75	1.25	2.50	10.0	2.96
E	2.50	1.25	3.75	2.50	10.0	2.88
F	3.75	1.25	2.50	2.50	10.0	2.76
G	1.25	3.75	2.50	2.50	10.0	2.88
H	1.25	2.50	2.50	3.75	10.0	2.95
I	3.75	2.50	2.50	1.25	10.0	2.76
A	1.25	1.25	1.25	1.25	5.00	2.10
B	1.25	1.25	2.00	0.500	5.00	2.09
C	1.25	1.25	0.500	2.00	5.00	2.14
D	1.25	2.00	0.500	1.25	5.00	2.30
E	1.25	0.500	2.00	1.25	5.00	2.37
F	2.00	0.500	1.25	1.25	5.00	2.36
G	0.500	2.00	1.25	1.25	5.00	2.18
H	0.500	1.25	1.25	2.00	5.00	2.24
I	2.00	1.25	1.25	0.500	5.00	2.13

Figure 8 shows the cyclic voltammograms response for three mixtures for two different total concentrations. The data show that regardless of the combination of the different concentration, the ratio is almost the same (standard deviation < 5%), indicating that in a mix the individual behaviour of each molecule does not significantly influence the amount output which is rather controlled by the total concentration of the species studies.

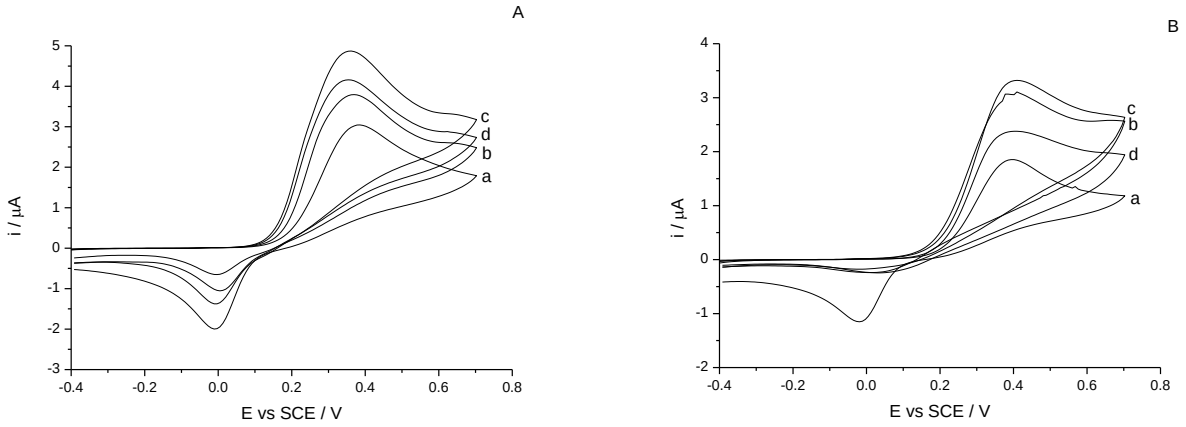


Figure 8. Cyclic voltammetric responses in PBS (pH = 7.5) in the absence (a) and presence of 40μM "mix" (A) and 80μM "mix" (B) using 0.3% catechol dissolved in nanocarbon paste electrode. Curves b, c and d: "mix" A, C and I, respectively.

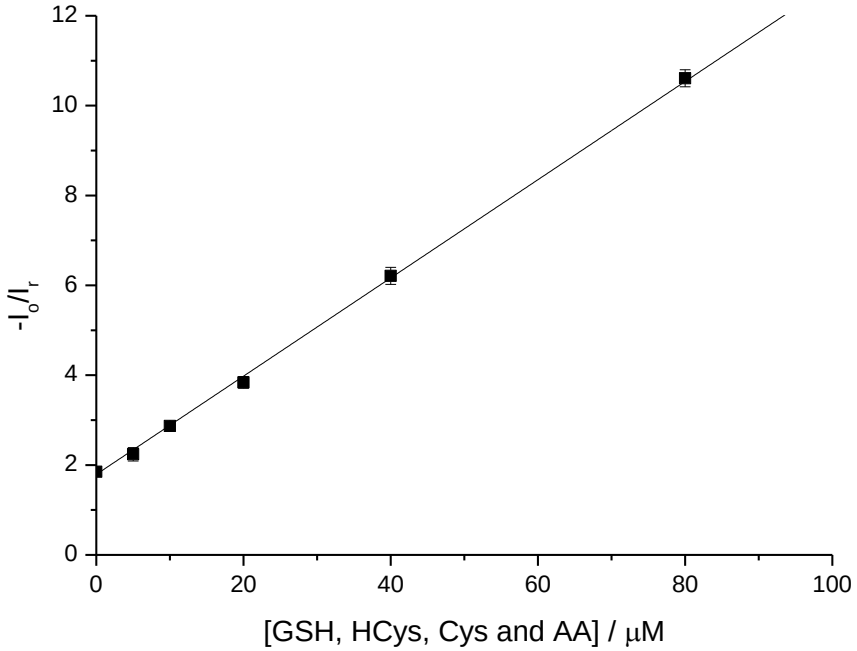


Figure 9. Calibration curve for total antioxidants using nanocarbon-catechol paste electrode.

Figure 9 shows the calibration curve for the total concentration of antioxidants. The linear equation obtained was -Io/Ir (μA) = 1.79 + 0.11[antioxidants] (μM). The reproducibility for each different total concentration was between 3-5% (n = 3) and the detection limit was 1.93μM, demonstrating the good performance of the modified electrode.

Table 4 shows the final comparison between the two systems. Both paste electrodes were efficient to quantify total antioxidants based in a reaction with electrochemically generated *o*-quinone. The nanocarbon-catechol paste electrode has the advantage that is a reagentless sensor in comparison of nanocarbon paste electrode but note it does need to be kept in nitrogen atmosphere to avoid catechol oxidation.

Table 4. Comparison between the nanocarbon paste and nanocarbon-catechol paste electrodes for total antioxidants detection.

Parameters	Nanocarbon paste	Nanocarbon-catechol paste
Store	Room temperature	Room temperature in N_2 atmosphere
Catechol	In solution: 100μM	In the paste: 0.3%
Total antioxidants - range	5 to 100μM	5 to 80μM
Sensitivity	$8.8x10^{-3}$μA/μM	$0.11μM^{-1}$
Detection limit	2.0μM	1.9μM

3.4 Antioxidant determination in multivitamin drug samples

After the detection of glutathione, cysteine, homocysteine and ascorbic acid using unmodified and modified nanocarbon paste electrode, both paste electrodes was used to determine total antioxidant concentrations in commercial multivitamin drug samples with different formulations. The results were obtained using both the above reported analytical curves: I_o (μA) = 2.409 + 0.0088[antioxidants] (μM) and $-I_o/I_r$ (μA) = 1.79 + 0.11[antioxidants] (μM), respectively, for unmodified and modified nanocarbon paste electrodes. Table 5 reports the results obtained with both paste electrodes compared to those expected as reported on the product. The good agreement between results shown in the table confirms the sensors (unmodified and modified) consist of a reliable methodology to determine antioxidants in multivitamins drugs samples.

Table 5. Antioxidants content in commercial multivitamin drug samples with different formulations.

Samples	AA / mol*	GSH / mol*	Cys / mol*	Total / mol*	Nanocarbon paste electrode / mol	Nanocarbon-catechol paste electrode / mol
1	$2.8x10^{-3}$	$6.5x10^{-6}$	$1.6x10^{-4}$	$3.0x10^{-3}$	$(3.3±0.1)x10^{-3}$	$(3.21±0.08)x10^{-3}$
2	$4.5x10^{-4}$	$3.2x10^{-5}$	-	$4.8x10^{-4}$	$(4.72±0.05)x10^{-4}$	$(4.7±0.1)x10^{-4}$
3	$5.1x10^{-4}$	$8.1x10^{-6}$	-	$5.2x10^{-4}$	$(5.1±0.2)x10^{-4}$	$(5.05±0.05)x10^{-4}$

AA = ascorbic acid; GSH = glutathione; Cys = cysteine

*reported on the product

4. CONCLUSIONS

In this report the application of nanocarbon paste electrodes for detecting total concentrations of glutathione, homocysteine, cysteine and ascorbic acid based on the reaction with electrochemically generated *o*-quinone was described. The use of nanocarbon paste electrodes was demonstrated by measurement the concentration of total antioxidants in multivitamin drug samples and the reliability of the method.

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