Article

Voltammetric Electrochemical Behavior of Carbon Paste Electrode Containing Intrinsic Silver for Determination of Cysteine

Maša Buljac [1,*], Denis Krivić [2], Ivana Škugor Rončević [3], Nives Vladislavić [3] and Marijo Buzuk [3]

1   Department of Environmental Chemistry, Faculty of Chemistry and Technology, University of Split, 21000 Split, Croatia
2   Division of Biophysics, Gottfried Schatz Research Center, Medical University of Graz, 8010 Graz, Austria; denis.krivic@medunigraz.at
3   Department of General and Inorganic Chemistry, Faculty of Chemistry and Technology, University of Split, 21000 Split, Croatia; skugor@ktf-split.hr (I.Š.R.); nives@ktf-split.hr (N.V.); buzuk@ktf-split.hr (M.B.)
*   Correspondence: masa@ktf-split.hr; Tel.: +385-21-329-479

Abstract: In this paper, the electrochemical behavior of cysteine is described, using carbon paste electrodes (CPEs) modified with ternary silver-copper sulfide containing intrinsic silver at two pH values (pH 3 and 5). Experiments have revealed that presence of cysteine has a large impact on the electrochemical behavior of modified CPEs. Observed phenomena take place in solution, as well as at the surface of the modified CPEs, and can be applied for electroanalytical purposes. Based on the electrochemical behavior observed in the examined system, differential pulse voltammetry (DPV) was selected as an electroanalytical method for determination of cysteine. The effects of the various parameters on the electroanalytical signal, such as the amount of electroactive material, electroanalytical parameters, pH etc., were investigated using differential pulse voltammograms. The results indicated that electrochemical signal characterized with well-defined cathodic peak at 0.055 V vs. Ag/AgCl (3 M) in acetic buffer solution at pH 5 can be used for indirect electrochemical determination of cysteine. The optimization procedure revealed that the most sensitive and stabile electrode was that containing 5% modifier. The DPV response of the electrode, in the presence of cysteine, showed two different linear concentration ranges of 0.1 to 2.5 μM, and 5.6 to 28 μM. The explanation of the origin of two linear ranges is proposed. The lower concentration range was characterized by remarkable sensitivity of the 11.78 μA μM^{-1}, owing to the chosen indirect method of determination. The calculated limit of detection (LOD), as well as limit of quantification (LOQ) were 0.032 and 0.081 μM, respectively. The influence of interfering agents on the electroanalytical response was examined, and low or no interference on the DPVs was observed. The proposed method was validated and applied for the determination of cysteine in pharmaceutical preparations with satisfactory recoveries in the range of 97 to 101.7%.

Keywords: electroanalysis; cysteine; differential pulse voltammetry; carbon paste electrodes; ternary sulphides; intrinsic silver

1. Introduction

Thiols, as components of various proteins and simple molecules, play an important role in many biological and environmental systems. Biological thiols such as cysteine, glutathione and homocysteine have attracted special attention because they are widely distributed in the environment, as intermediates important in the anaerobic decomposition of organic matter in sediments and soil. These three particular thiols share a similar structure, which has been a major obstacle for researching their physiological functions [1].

Cysteine is a sulfur-containing non-essential amino acid with pK_a values of 1.96 for the carboxylic group, 8.18 for the amine group and 10.28 for the thiol group [2]. Because it

contains a strongly nucleophilic thiol group, cysteine is an interesting target for bioassays aimed at investigating the biomolecular structure and dynamics of reactions [3]. Specifically, this thiol group enables cysteine to interact with more complex biological molecules, in that it creates strong bonds with the silver or gold electrode, and amino and carboxyl groups [4]. A particularly interesting interaction of cysteine with silver surfaces occurs, that indicates a stronger adsorption of cysteine on silver than on copper or gold surfaces. Such interactions may affect the creation of new interface states at the cysteine–Ag interface [5]. Silver(I) ion forms strong complexes with organic and inorganic sulfur from thiols, which is indicated by a higher stability constant for Ag(I) organosulfur complexes (thiols, $K \approx 10^{13}$), compared to those of Ag(I) carboxylate complexes (monodicarboxylic acids, $K \approx 10^2$–10^4, or EDTA with $K \approx 10^7$) [6]. Possible binding of cysteine on the surface of Ag nanoparticles was reported by Ravindran et al. [2].

Toh et al. reported chemical interactions between silver nanoparticles and thiols, showing how silver (I) forms complexes with inorganic and organic thiols without redox reactions [7]. They also described the effect of pH on the binding of silver nanoparticles to cysteine in terms of preventing aggregation of silver nanoparticles. The effect of Au NPs on cysteine electroanalysis was investigated by Wang et al., who studied the sensitive detection of cysteine performed on a modified GC electrode with Au nanoparticles dispersed in Nafion [8]. Pei et al. reported electrochemical behavior of metal-based sulfides (Ag$_2$S and Ag$_2$S/Cu$_2$S) in the presence of cysteine, demonstrating that the glassy carbon electrode modified with Ag$_2$S or Ag$_2$S/Cu$_2$S shows semi-reversible behavior, which suggests that such prepared electrodes have potential for cysteine determination [9]. In our previous paper we focused on the investigation of the electrochemical behavior of cysteine on different crystal electrodes based on silver-copper sulfides. Electrode material comprising metallic silver and jalpaite showed the best electrochemical behavior towards cysteine [10]. A number of studies have shown that silver is a good substrate for electrodes for the selective determination of thio compounds, in the range of submicromolar concentrations, at low applied potential [11].

There are many questions among researchers about the electrooxidative mechanism of amino acids, and the scientists generally agree that it is vastly dependent on experimental conditions such as variations in pH and electrolyte, type of electrode, and amino acid concentration itself [12].

Accordingly, many chemical and instrumental methods have been reported for the determination of cysteine. These include chromatography separation [11,13,14], spectrophotometry [2,5,7,15] and electrochemical techniques [15–18]. Compared to other methods, electroanalytical approaches have inherent advantages such as simplicity, high sensitivity, good stability and relatively low price.

The electrochemical behavior and sensitive detection of cysteine can be determined by various electrochemical techniques, including cyclic voltammetry, differential pulse voltammetry and cathodic stripping voltammetry [16,19–21]. Several electrodes usually used for the investigation of cysteine include glassy carbon electrodes or modified glassy carbon electrodes [16,19,22], carbon paste electrodes or modified carbon paste electrodes [17,20,23] and mercury electrodes [24].

Comparison of the electroanalytical performances of electrodes modified with various materials for the determination of cysteine, obtained from papers published in the last five years, are given in Table 1. It should be noted here that in this period there have been very few papers describing the determination of cysteine with electrodes modified with various silver-based materials.

Table 1. Comparison of the electroanalytical performance of electrodes modified with various materials, for determination of cysteine.

Supporting Electrode/Sensitive Layer	pH	Working Potential E/V	LOD/µM	Linear Range µM	Sensitivity	Method	Ref.
GCE/AgNPs/GQDs	7	0.52 V vs. SCE	0.01	0.1–5; 8–200	NA	CV, DPV	[19]
silver-copper sulfide	5 7	0.142 V vs. Ag/AgCl 0.04 V vs. Ag/AgCl	0.036 0.024	1–100	0.11 µA µM^{-1} 0.10 µA µM^{-1}	AMP	[10]
GCE/rGO-Nafion@Pd	7	0.6 V vs. SCE	0.15	0.5–10	1.30 µA µM^{-1} cm^{-2}	AMP	[25]
screen-printed diamond electrode	7	0.663 V vs. Ag/AgCl	0.872	1–194	0.226 µA µM^{-1} cm^{-2}	CV	[26]
N-PC/Ag-MOF	7	0.02 V vs. Ag/AgCl	0.05	0.1–1300	NA	AMP	[27]
CuO/boron nitride Nanocomposite	7.4	0.45 V vs. Ag/AgCl	0.58	1–10	NA	AMP	[28]
functionalized MWCNT	7	0.2 V vs. SCE	0.16 nM	0.7 nM–200µM	NA	DPV	[29]
CeO$_2$-CuO nanocomposite	7	0.8 V vs. SCE	0.016	10–5000	21.6 µA cm^{-2} mM^{-1}	AMP	[30]
CeO$_2$-SnO$_2$ nanocomposite	7	0.8 V vs. SCE	16	10–2000	186.34 µA cm^{-2} mM^{-1}	AMP	[31]
CuFe$_2$O$_4$/rGO–Au composite	6.5	0.4 V vs. Ag/AgCl	0.383	50–200	100.01 µA cm^{-2} mM^{-1}	CV	[32]
ethyl 2-(4 ferrocenyl[1,2,3]triazol-1-yl) acetate/graphene	7	0.33 V vs. Ag/AgCl	0.9	4.0–2300.0	0.0078 µA µM^{-1}	SWV	[33]
Au nanoparticles/ anthraquinone-2-carboxylic acid	7	0.8 V vs. SCE	1.873	15–500	NA	DPV	[34]
electrodeposited copper/SPE	7.4	0.5 vs. Ag pseudo-ref	0.21	1–400 400–1800	0.028 µA µM^{-1} 0.014 µA µM^{-1}	AMP	[35]
Cu^{2+} modified Fe$_3$O$_4$@polydopamine	5	0.1 V vs. Ag/AgCl	83.0 pM	0.010–500	102 µA µM^{-1} cm^{-2}	SWV	[36]
Co-Gd$_2$O$_3$ nanocomposite	7	0.4 V vs. Ag/AgCl	0.23	1–100	NA	AMP	[37]
bismuth tellurate nanospheres	7	0.14 V vs. SCE	0.046	0.1–2000	NA	CV	[38]
CPE/ternary silver-copper sulfides	5	0.055 V vs. Ag/AgCl	0.032 0.08	0.1–2.5 5.6–28	11.78 µA µM^{-1}	DPV	This work

GCE: glassy carbon electrode, MWCNT: multiwall carbon nanotubes, SPE: screen printed electrode, CPE: carbon paste electrode. Methods—AMP amperometry, CV cyclic voltammetry, DPV differential pulse voltammetry, SWV squarewave voltammetry.

Our previous paper presented the use of a carbon paste electrode modified with ternary silver-copper sulfides as an amperometric sensor for indirect determination of hydrogen peroxide [39]. Relying on these results, in this paper we focused on the electrochemical behavior of cysteine on a carbon paste electrode modified with silver-copper sulfide materials. In this work, an electrochemical indirect method for determination of cysteine is presented. This indirect method relied on the chemical complexation of cysteine with silver ions. As a source of silver ions, ternary silver-copper sulfide containing intrinsic silver was used, incorporated in the carbon paste electrode. As an appropriate method, differential pulse voltammetry was chosen. Owing to the indirect method, excellent sensitivity at low cysteine concentration was achieved. The influence of pH and different content of electroactive material was investigated.

2. Materials and Methods

2.1. Chemical and Solution

All reagents and chemicals were of analytical grade, and all solutions were prepared with redistilled water. Acetate buffer (pH 3 and 5; 0.1 M) was prepared by mixing solutions of 0.1 M acetic acid and 0.1 M potassium acetate, all purchased from Kemika (Zagreb, Croatia). The cysteine solution (Merck, Kenilworth, NJ, USA) and pharmaceutical preparations (NOW Foods tablets (dietary supplement)—NOW FOODS, Bloomingale, Illinois, USA; Fluimukan Akut effervescent tablets—Sandoz, Zagreb, Croatia and Propomucil ® throat syrup—Abela Pharm, Belgrade, Serbia) were prepared daily by dissolution in previously deaerated (with N_2) redistilled water. This solution was used for the preparation of solutions with lower cysteine concentrations. Arginine, histidine, ascorbic acid and aspartic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA), while lactose was obtained from Kemika (Zagreb, Croatia).

2.2. Preparation of Modified Electrode

Electrode materials were obtained by co-precipitation of Ag^+ and Cu^{2+} with sulfide excess of metals ions, as shown in Table 2.

Table 2. Mole ratio of the copper, silver and sulfide ions.

Composition of Mediator	Mole Ratio of Ions	Moles of Ions
metallic silver, $Ag_{1.2}Cu_{0.8}S$ and CuAgS	$(Cu^{2+}; Ag^+): S^{2-}$ 1:0.5	$Cu^{2+}; Ag^+; S^{2-}$ 0.02; 0.04; 0.02

Modified carbon paste electrodes (CPEs) were used as the working electrodes. The modified CPE containing 5% by weight of the material were prepared by hand-mixing 0.475 g of graphite powder (<20 µm, Sigma-Aldrich, St. Louis, MO, USA) and 0.025 g (5% by weight) of modifier. The mixture of graphite powder and mediator was used for preparation of carbon paste with the addition of 200 mm³ of paraffin oil (Kemika, Zagreb, Croatia) followed by homogenization in a mortar. Similar procedures were applied for CPEs with 2.5% and 10% of mediators. Obtained pastes were kept overnight in the refrigerator. CPEs were prepared by packing prepared paste into a syringe with a working surface of 0.2 cm². Electrical contact was made by inserting a copper wire into the paste. The working surface of the electrode was polished with a wet filter paper. The mediator was ternary silver–copper mixed sulfide with composition: metallic silver, $Ag_{1.2}Cu_{0.8}S$ and CuAgS; composition of the mediator was determined by XRD analysis [40]. This mediator was synthesized according to the proposed method [10,40] in the presence of an excess of the metal ions. The mole ratio and molarity details of the ions in the preparation procedure are given in Table 2.

2.3. Apparatus and Measurements

All experiments were carried out in an electrochemical cell with three electrodes, at room temperature (ca. 22–25 °C). Prepared modified CPEs were used as the working electrodes, a Radiometer XR300 Ag/AgCl 3 M electrode (Copenhagen, Denmark) was used as a reference electrode, and platinum as an auxiliary electrode. Electrochemical measurements (cyclic voltammetry and differential pulse voltammetry) were carried out using a potentiostat (Autolab PGSTAT 302N, Metrohm, Autolab B.V., Utrecht, The Netherlands), connected to a PC and driven by GPES4.9 software (Eco Chemie, Metrohm, Autolab B.V., Utrecht, The Netherlands).

Cyclic voltammograms were recorded in the range of −0.25 to +0.75 V, in quiescence solution at a scan rate of 50 mV s⁻¹, using modified CPEs in 50 cm³ acetate buffer (pH 3 and 5), unless otherwise stated.

Differential pulse voltammograms (DPV) were carried out in 50 cm³ acetate buffer (pH 5) spiked with aliquots of cysteine to obtain desired cysteine concentrations. As in the case of CV, prior to cysteine addition, the electrode was cycled 10 times with a scan

rate of 25 mV s^{-1}. The differential pulse voltammetry (DPV) was applied under optimized parameters, i.e., pulse amplitude 0.05 V, pulse width 0.05 s and step potential 0.01 V. The solution was stirred and then left to rest for 15 s before each voltammetric measurement.

3. Results and Discussion

3.1. Composition of the Modifier

Phase composition of the synthesized material was determined by x-ray powder diffraction (XRD), as previously reported [11,40]. The pattern for the material clearly indicates the existence of the metallic silver and silver-copper sulfides ($Ag_{1.2}Cu_{0.8}S$ and CuAgS).

3.2. Cyclic Voltammetry

The pH values were chosen with respect to pK_a values of the cysteine; in the pH range 1.92–8.37 cysteine exists as zwitterion. Furthermore, presence of the intrinsic metallic silver in the modifier restricted higher pH values, at which formation of hydroxo-species of the silver can occur.

Thus, the electrochemical behaviors of electrodes consisting of various amounts of material were observed in acetic buffer solution at pH 3 and 5. The results of cyclic voltammetry and recorded cyclic voltammograms (CVs) are presented in Figure 1. All recorded CVs gave obvious cathodic and anodic current peaks. Both peaks are a consequence of the redox behavior of intrinsic silver, present in the material.
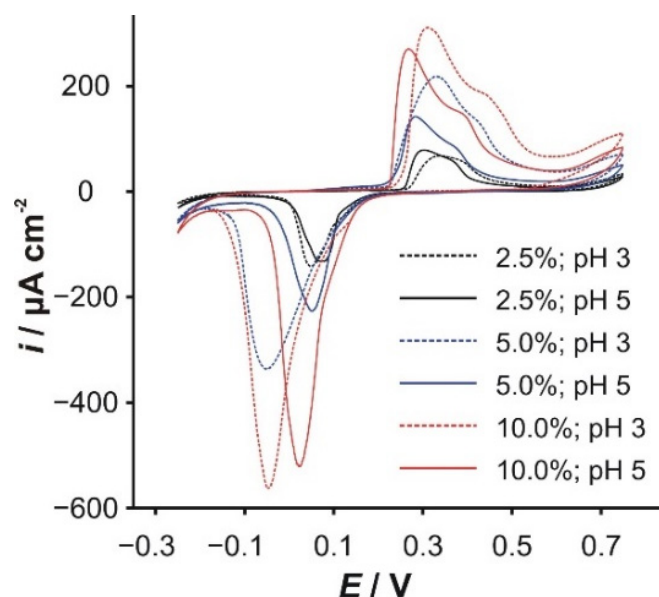

Figure 1. Cyclic voltammograms of the CPEs, modified with various amount of the material, recorded at the different pH values (0.1 M acetate buffers).

This claim is supported by CV recorded upon addition of 10^{-5} M silver ions, at electrode modified with 5% of material at pH 5. As shown, current peak was more pronounced at the same potential in the presence of the silver ions. Furthermore, we investigated influence of the addition of cupric ions, which are also released from the material, according to Equation (1). Meager influence on the current peaks was found when compared to the addition of silver ions.

Generally, both current peaks were higher at the lower pH value (pH 3). This behavior is not surprising if we take into consideration a simplified reaction that can be applied on both types of copper-silver sulphides:

$$CuAgS + H^+ \rightleftharpoons Cu^{2+} + Ag^+ + HS^- + e^- \tag{1}$$

Somewhat improved dissolution occurred at lower pH, besides the release of the silver ions from the copper–silver material, also as consequence providing more available intrinsic silver. Consequently, both current peaks were higher. This effect was followed by larger separation between oxidation and reduction peaks. As expected, higher amounts of the materials in CPEs produced larger currents.

As we shall discuss later, mechanism of determination of cysteine relies on the complexation of the cysteine with silver ions. As a consequence of the higher amount of available silver ions, a lower current response would be expected at lower cysteine concentrations.

Referring to the above-mentioned behavior, and taking into account the possibility of determining cysteine in real samples at more "natural" pH values, a decision was made to further proceed with investigation at pH 5.

Influence of the scan rate on the CVs was examined in order to gain insight into problems related to the mechanism of both redox processes. Recorded CVs for electrode modified with 5% of the material is presented in Figure 2. Analyses of the cathodic current peaks high in dependence on the scan rate, square root of the scan rate, and $\log(v) - (-\log|i|)$ dependence for all prepared electrodes are shown in Figure 3.
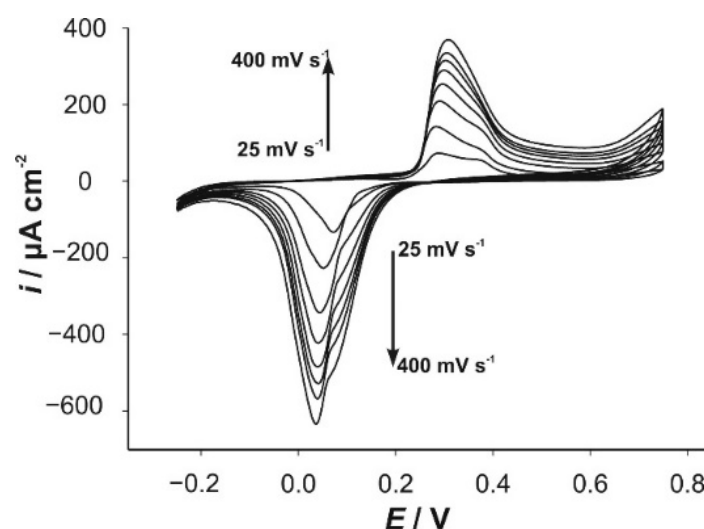

Figure 2. Cyclic voltammograms recorded in 0.1 M acetate buffer solution at pH 5, with the CPE modified with 5% of the material, at different scan rates: 25, 50, 100, 150, 200, 250, 300 and 400 mV s^{-1}.

The obtained dependences (Figure 3a,b) reveal that cathodic reaction is governed by the diffusion process, since values of the current peaks high in dependence on the square root of scan rate are linear in large parts of the applied scan rates. However, the best linearity was obtained using electrode with 5% of material. Additional proof of the above-mentioned is revealed from dependence of the $\log(v) - (-\log|i|)$ (Figure 3c). As presented, all electrodes show linearity in some part with the slope of 0.55, which is close to the theoretic slope for diffusion-controlled processes. Accordingly, based on the previous results, the electrode with 5% of the materials was selected for further work and optimization.

In addition, beside analysis of the cathodic reaction, analysis was also performed of the behavior of the anodic peak currents according to the scan rate. The recorded CVs for the electrodes modified with 2.5% and 5.0% of the material are presented in Supplementary Figure S1, while corresponding analysis of the anodic current peaks vs scan rates and square root of the scan rates are given in Supplementary Figure S2. As presented, no linearity was observed in the dependences of the anodic current peaks on the scan rate, nor of the square roots on scan rates. In fact, anodic current peaks tended towards constant values with increase of the scan rate. This suggests a somewhat complex mechanism that involves diffusion phenomena as well as surface-related phenomena. Further confirmation for this can be found in the CVs presented in Supplementary Figure S3. At a scan rate of

25 mV s^{-1}, the width of the anodic peak was proportional to the content of the material, suggesting that the anodic peak was a consequence of the silver reduced from the previous cycle as well as intrinsic silver from the material, which produced the complex form of the anodic peak. Whatever the reason, the complex mechanism of the anodic process is not suitable for electroanalytical purposes.
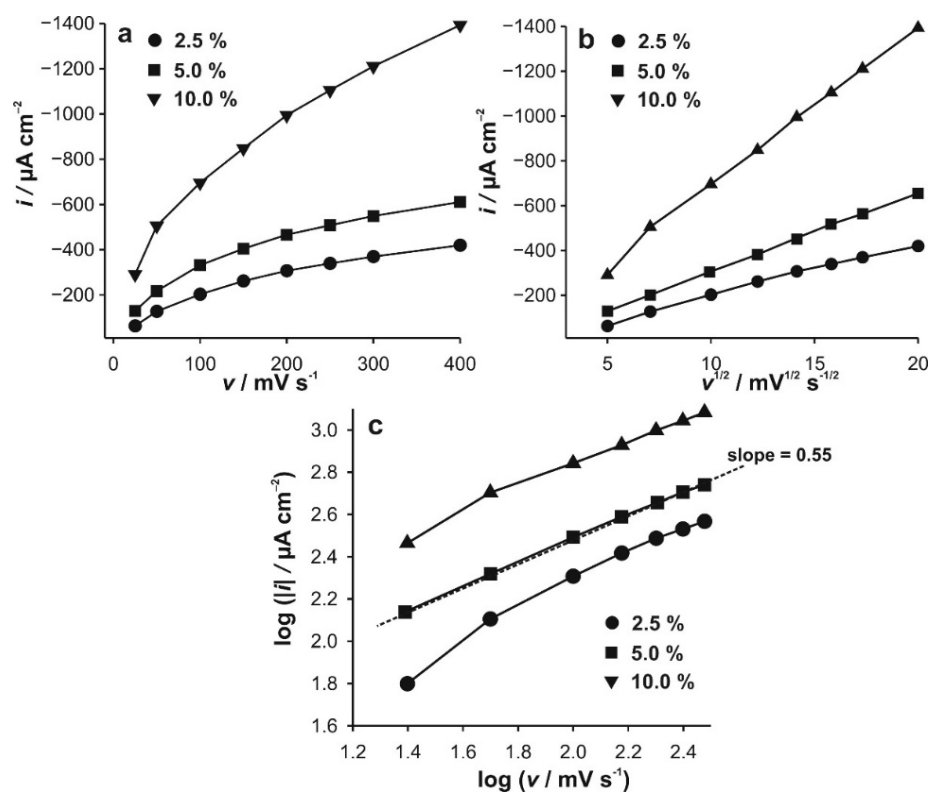

Figure 3. Dependences of the cathodic peak currents for the CPEs modified with different amounts of materials on: (**a**) scan rate; (**b**) square root of scan rate; (**c**) logarithm of oxidative current peak vs logarithm of scan rate. The dependences were obtained from the cyclic voltammograms presented in Figure 2.

The cyclic voltammograms recorded for electrode modified with 5% material in the presence of cysteine are presented in Figure 4a.
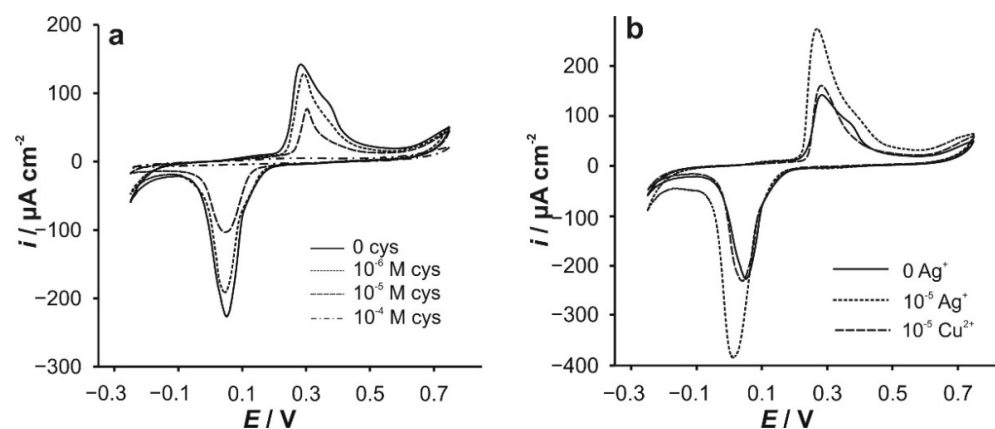

Figure 4. (**a**) Cyclic voltammograms recorded with CPE, modified with 5% of the material, without and in the presence of: (**a**) cysteine (successive addition of cysteine); (**b**) 10^{-5} M of silver ions or cupric ions. All presented CVs were recorded in acetate buffer solution at pH 5, at a scan rate of 50 mV s^{-1}.

As shown, both current peaks decreased as the cysteine concentration increased. A decrease of both of the peaks was followed by no shift in the potentials of the current peaks. This indicates that the phenomenon arose from the lower concentration of silver ions. This lower concentration was a consequence of the complexation of the cysteine with silver ions; it is well known that thiols tend to react with silver ions to form stable, undissociated, mercaptides [18,41].

A decrease of both current peaks took place at cysteine concentration ranges between 0.1 and 10 μM. At higher concentrations of cysteine, the electrode become polarizable in the measured potential range. This phenomenon can be attributed to the adsorption of the products of the complexation onto the electrode surface, causing the electrode to become blocked.

3.3. Differential Pulse Voltammetry (DPV)

Taking into account everything mentioned above, differential pulse voltammetry (DPV) was chosen as a suitable method for electroanalytical determination of the cysteine at given conditions.

DPVs recorded at electrode modified with 5% of material in the presence of the various concentrations of cysteine are presented in Figure 5. The dependences of the current peak on the cysteine concentration, produced by DPVs, are presented in Figure 5.
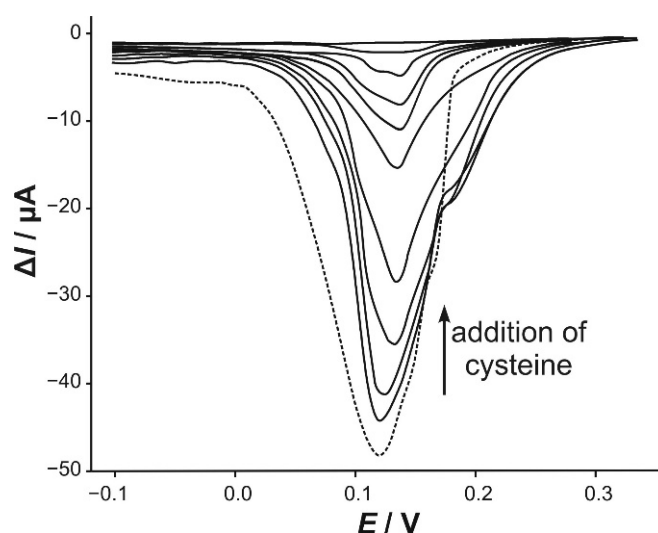

Figure 5. Differential pulse voltammograms, recorded at the CPE modified with 5% of the material, in acetate buffer at pH 5, upon successive addition of cysteine for obtaining: 0.1, 0.4, 0.9, 1.6, 2.6, 5.6, 10, 17, 27, 57, 100 and 170 μM in an acetate buffer solution (pH 5). The dashed line represents DPV recorded without cysteine solution. Initial measurement parameters: conditional potential 25 mV, pulse amplitude 0.05 V, pulse width 0.05 s, step potential 0.01 V.

As shown in Figure 6, during the determination of cysteine, two different linear concentration ranges of cysteine were observed, 0.1 to 2.5 μM, and 5.6 to 28 μM.

Analytical response in lower concentration ranges is characterized with good linearity (R^2 = 0.998) and excellent sensitivity of 11.78 μA μM^{-1}. This was expected, considering preliminary results obtained by CV and presented in Figure 4a. It is obvious that in this concentration range there is negligible influence on adsorption of the products of complexation at the electrode surface. The decrease of the slope (0.45 μA μM^{-1}) in concert with preservation of linearity can to some extent be attributed to the adsorption of complexation products at the electrode surface. This decrease in linearity was followed by an increase in the peak width ($w_{1/2}$). At higher concentrations of cysteine, DPVs become more complex in shape, indicating a change in the mechanism of silver reduction, probably related to the reduction from the complex and/or the EC-controlled mechanism (decomposition of the complex followed by electrochemical reduction of silver). However, this did not have an

impact onto electroanalytical capabilities, and the reproducibility of the analytical signal was unaffected (see error bars). The repeatability and reproducibility of the electrode was investigated, and relative standard deviation of five-value datasets did not exceed 3%. It must be emphasized that prior to repeatability measurements, the electrode surface was renewed by removing the surface layer, followed by cyclization between 0.3 V and 0.7 V at a scan rate of 25 mV s^{-1} for 10 cycles. This cyclization ensured an optimal amount (see Section 3.2) of the silver ions in solution for complexation with cysteine.
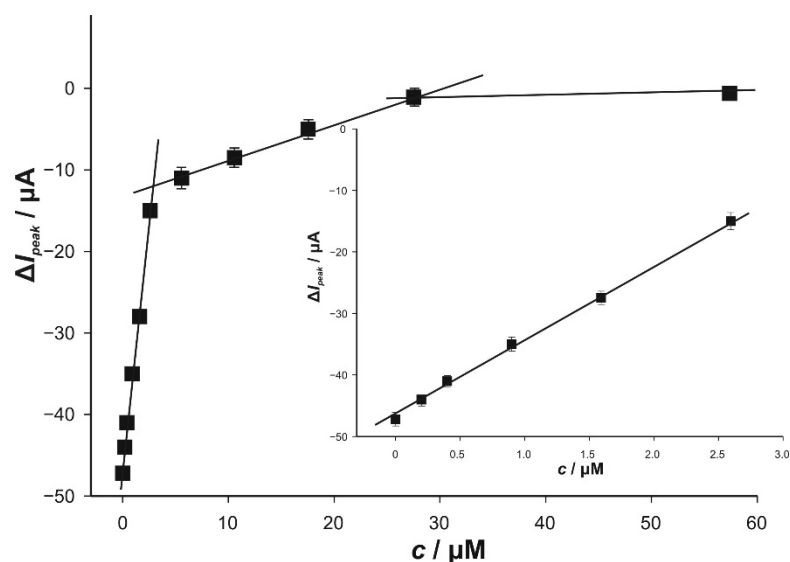

Figure 6. The calibration plot of the cathodic current peaks dependent on cysteine concentration (sequential addition of cysteine), derived from voltammograms presented in Figure 5. The inset represents the lower concentration range. For the plot, the five data value sets were used.

As an important part of the development of electroanalytical methods, optimization of DPV parameters were performed. Parameters including modulation time (t_{DP}), modulation amplitude (E_{DP}) and step potential (ΔE_s) did not significantly affect electroanalytical performability. However, it was found that conditional potential (E_{CP}) had an impact on electroanalytical signals. The results of this impact are presented in Figure 7.
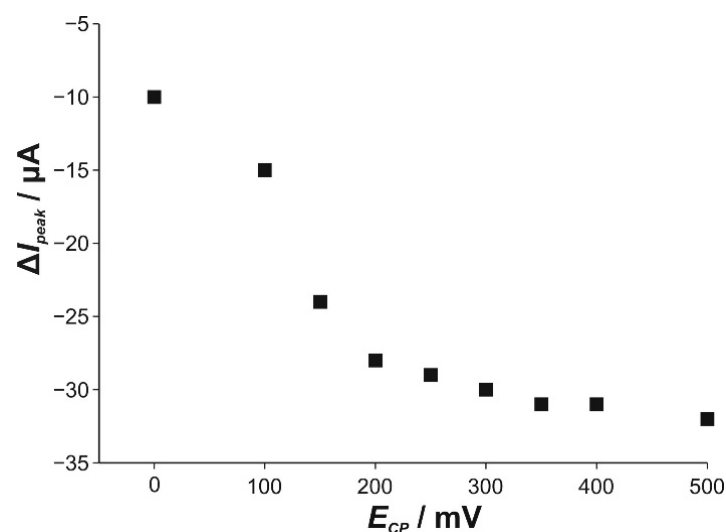

Figure 7. The effect of the conditional potentials on the DPV signal. DPVs were recorded with CPE modified with 5% of material in the presence of 17 μM of cysteine at pH 5. Other measurement parameters: conditional time 20 s, pulse amplitude 0.05 V, pulse width 0.05 s, step potential 0.01 V.

As shown, conditional potentials higher than 250 mV had a low effect on current. Accordingly, conditional potential E_{CP} = 250 mV was chosen for 20 seconds as current signal was unaffected for conditional potential time (t_{CP}) above 15 s.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to IUPAC definitions and recommendations [42] using the signal to-noise (S/N) method. S/N ratios of 10:1 and 3:1, which approximate the requirements for LOQ and LOD, were determined as 0.081 and 0.032 µM, respectively.

3.4. Effects of Interfering Compounds

Under optimum conditions, the selectivity of prepared CPE for determination of cysteine was examined using several potential silver-interfering compounds, including amino acids arginine, histidine and aspartic acid. The DPV was carried out in the presence of cysteine (2.6 µM) and similar concentrations of interfering compounds.

Previous studies report that basic amino acids arginine and histidine are the strongest Ag+ binders, with structures similar to cysteine, but they do not have -SH groups. Aspartic acid is a more complex system because it has two carboxyl groups and it can be considered both an α-and β-amino derivative [43,44].

According to the obtained results (Figure 8), no significant interfering effects were observed, while only cysteine (amino acid with thiol group) could bind silver.
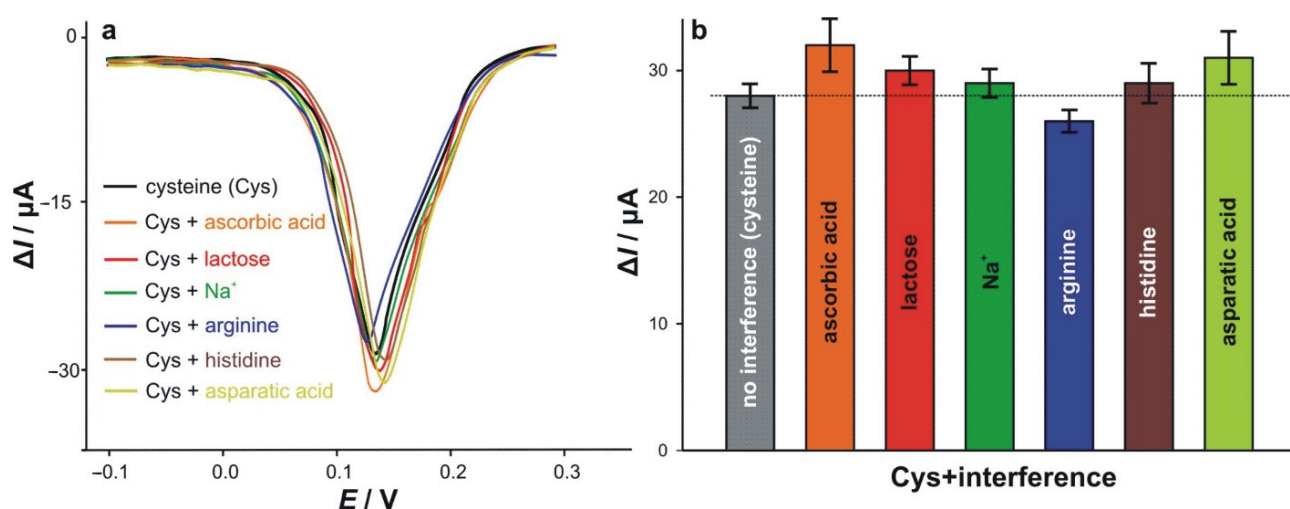

Figure 8. (**a**) The DPV responses of cysteine in presence of potential interference; (**b**) Interference signal of Cys with AA, lactose, Na$^+$, Arg, His and Asp.

These results suggest that the selectivity of the selected electrode is superior to other amino acids for the detection of cysteine. Similar behaviors described in articles by Han et al. and Borase et al. [45,46].

Furthermore, the interference effect of certain ions and species labeled in pharmaceutical preparations, such as sodium, lactose and ascorbic acid was also investigated. In this case, no significant interference was observed, which indicates the possibility of using this electrode for the determination of cysteine in selected real samples by the electrochemical method based on differential pulse voltammetry, under optimized conditions.

3.5. Detection of Cysteine in Real Samples

In order to confirm the claim made above, the applicability of the electrode to cysteine determination was investigated using the standard addition method for selected real samples, i.e. pharmaceutical preparations, including NOW Foods tablets (dietary supplement), Fluimukan Akut effervescent tablets and Propomucil ® throat syrup.

The obtained results of three replicate measurements are shown in Table 3.

Table 3. Determination of cysteine in real samples by using standard addition method.

Sample	Expected/μM	Found/μM	Average/μM	RSD/%	Recovery/%
NOW Foods tablets	1	0.96; 0.95; 1.02	0.98	3.9	97.7
Fluimukan Akut	6	6.13; 6.23; 5.94	6.10	2.4	101.7
Propomucil	24	23.91; 24.32; 24.15	24.13	0.9	100.5

The recoveries varied from 97.7 to 101.7% and relative standard deviations were less than 3.9% ($n = 3$). The obtained results showed that the proposed DPV method was suitable for cysteine determination in real samples.

4. Conclusions

This paper presents the electrochemical behavior of CPEs modified with different amounts of silver–copper sulfides containing intrinsic silver, in order to determine possible electroanalytical application for determination of cysteine. Differential pulse voltammetry (DPV) was selected as the electroanalytical method for determination of cysteine, based on the electrochemical behavior observed in the examined system. The effects on differential pulse voltammograms of various parameters of the electroanalytical signal, such as the amount of electroactive material, electroanalytical parameters, pH, etc., were investigated. The results revealed that the optimal electrochemical behavior was obtained using electrode modified with 5% of material, with well-defined cathodic peak at 0.055 V vs Ag/AgCl (3 M), in acetic buffer solution at pH 5. As an analytical signal, the decrease of cathodic current peak is attributed to the decrease of silver ions near the electrode surface due to their complexation with added cysteine. This indirect method was characterized by a remarkable sensitive signal of the 11.78 μA μM^{-1} in the presence of low cysteine concentrations. The existence of the second linear range at a higher concentration of cysteine is attributed to the adsorption of the products of the complexation, and had no significant effect on reproducibility and repeatability. The calculated limit of detection (LOD) and limit of quantification (LOQ) were 0.032 and 0.081 μM, respectively. On the basis of the results obtained, it is apparent that the modified carbon paste electrode for determination of electrochemical behavior of cysteine showed good stability, short analysis time, high sensitivity and reproducibility. Finally, the proposed procedure was successfully validated using a standard addition method and applied in the determination of cysteine in pharmaceutical preparations including NOW Foods tablets (dietary supplement), Fluimukan Akut effervescent tablets, and Propomucil® throat syrup.

Author Contributions: Conceptualization, M.B. (Maša Buljac) and M.B. (Marijo Buzuk); methodology, M.B. (Maša Buljac) and M.B. (Marijo Buzuk).; validation, M.B. (Marijo Buzuk), D.K. and M.B. (Maša Buljac); formal analysis, N.V. and I.Š.R.; investigation, M.B. (Maša Buljac), M.B. (Marijo Buzuk); resources, M.B. (Maša Buljac); writing—original draft preparation, M.B. (Maša Buljac); writing—review and editing, M.B. (Maša Buljac), M.B. (Marijo Buzuk) and D.K.; visualization, M.B. (Maša Buljac); supervision, M.B. (Marijo Buzuk). All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xu, G.; Tang, Y.; Lin, W. A multi-signal fluorescent probe for discrimination of cysteine/homocysteine, and glutathione and the application in living cells and zebrafish. *New J. Chem.* **2018**, *42*, 12615–12620. [CrossRef]
2. Ravindran, A.; Dhas, S.P.; Chandrasekaran, N.; Mukherjee, A. Differential interaction of silver nanoparticles with cysteine. *J. Exp. Nanosci.* **2013**, *8*, 589–595. [CrossRef]
3. Chen, L.C.; Ho, K.C. Multimode optoelectrochemical detection of cysteine based on an electrochromic Prussian blue electrode. *Sens. Actuators B Chem.* **2008**, *130*, 418–424. [CrossRef]
4. Santos, E.; Avalle, L.; Pötting, K.; Vélez, P.; Jones, H. Experimental and theoretical studies of l-cysteine adsorbed at Ag(111) electrodes. *Electrochim. Acta* **2008**, *53*, 6807–6817. [CrossRef]
5. Koswattage, K.R.; Ishii, O. Photoemission investigation of interaction between L-cysteine and silver surface. *Surf. Interface Anal.* **2020**, *52*, 513–517. [CrossRef]
6. Bell, R.A.; Kramer, J.R. Structural chemistry and geochemistry of silver–sulfur compounds: Critical review. *Environ. Toxicol. Chem.* **1999**, *18*, 9–22. [CrossRef]
7. Toh, H.S.; Batchelor-Mcauley, C.; Tschulik, K.; Compton, R.G. Chemical interactions between silver nanoparticles and thiols: A comparison of mercaptohexanol against cysteine. *Sci. China Chem.* **2014**, *57*, 1199–1210. [CrossRef]
8. Wang, X.; Zhang, L.; Miao, L.; Kan, M.; Kong, L.; Zhang, H. Oxidation and detection of L-cysteine using a modified Au/Nafion/glass carbon electrode. *Sci. China Chem.* **2011**, *54*, 521–525. [CrossRef]
9. Pei, L.Z.; Yang, L.J.; Wang, J.F.; Fan, C.G.; Hu, J.L. Synthesis and Electrochemical Properties of Ag_2S and Ag_2S/Cu_2S Crystals. *e-J. Surf. Sci. Nanotech.* **2010**, *8*, 384–387. [CrossRef]
10. Vladislavić, N.; Škugor Rončević, I.; Buljac, M.; Brinić, S.; Krivić, D.; Buzuk, M. Electroanalytical Determination of Cysteine Using the Electrodes Based on Ternary Silver-Copper Sulfides. *Sensors* **2018**, *18*, 3753. [CrossRef]
11. Aydogmus, Z.; Sarakbi, A.; Kauffmann, J.M. Determination of Thiols and Free Thiol Content in a Protein with Liquid Chromatography Coupled to Amperometric Detection at a Silver Based Carbon Paste Electrode. *Electroanalysis* **2016**, *28*, 2703–2708. [CrossRef]
12. Moulaee, K.; Neri, G. Electrochemical Amino Acid Sensing: A Review on Challenges and Achievements. *Biosensors* **2021**, *11*, 502. [CrossRef]
13. González-García, O.; Ariño, C.; Díaz-Cruz, J. Comparison of voltammetric detection assisted by multivariate curve resolution with amperometric detection in liquid chromatographic analysis of cysteine-containing compounds *J. Chromatogr. A* **2005**, *1062*, 95–101. [CrossRef]
14. Alwael, H.; Connolly, D.; Barron, L.; Paull, B. Development of a rapid and sensitive method for determination of cysteine/cystine ratio in chemically defined media. *J. Chromatogr. A* **2010**, *1217*, 3863–3870. [CrossRef]
15. Hou, X.Y.; Chen, S.; Tang, J.; Long, Y.F. Visual determination of trace Cysteine based on promoted corrosion of triangular silver nanoplates by sodium thiosulfate. *Spectrochim. Acta A Mol. Biomol.* **2014**, *125*, 285–289. [CrossRef]
16. Liu, S.; Dai, G. Preparation and Electrochemical Behaviour of Silver Pentacyanonitrosylferrate Film Modified Glassy Carbon Electrode and Its Electrocatalytic Oxidation to L-cysteine. *J. Chin. Chem. Soc.* **2011**, *58*, 617–622. [CrossRef]
17. Raoof, J.B.; Ojani, R.; Beitollahi, H.; Hosseinzadeh, R. Electrocatalytic Oxidation and Highly Selective Voltammetric Determination of L-Cysteine at the Surface of a 1-[4-(Ferrocenyl ethynyl)phenyl]-1-ethanone Modified Carbon Paste Electrode. *Anal. Sci.* **2006**, *22*, 1213–1220. [CrossRef]
18. White, P.C.; Lawrence, N.S.; Davis, J.; Compton, R.G. Electrochemical Determination of Thiols: A Perspective. *Electroanalysis* **2002**, *14*, 89–98. [CrossRef]
19. Yao, J.; Liu, C.; Liu, L.; Chen, M.; Yang, M. An Electrochemical Sensor for Sensitive Determination of L-cysteine and Its Electrochemical Kinetics on AgNPs/GQDs/GCE Composite Modified Electrode. *J. Electrochem. Soc.* **2018**, *165*, 551–558. [CrossRef]
20. Raoof, J.B.; Ojani, R.; Kolbadinezhad, M. Differential Pulse Voltammetry Determination of L-Cysteine with Ferrocene-Modified Carbon Paste Electrode. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 818–826. [CrossRef]
21. Baldrianova, L.; Agrafiotou, P.; Svancara, I.; Vytras, K.; Sotiropoulos, S. The determination of cysteine at Bi-powder carbon paste electrodes by cathodic stripping voltammetry. *Electrochem. Commun.* **2008**, *10*, 918–921. [CrossRef]
22. Zhou, M.; Ding, J.; Guo, L.; Shang, Q. Electrochemical Behavior of L-Cysteine and Its Detection at Ordered Mesoporous Carbon-Modified Glassy Carbon Electrode. *Anal. Chem.* **2007**, *79*, 5328–5335. [CrossRef]
23. Raoof, J.B.; Ojani, R.; Zarei, E. Preparation of poly N,N-dimethylaniline/ferrocyanide film modified carbon paste electrode: Application to electrocatalytic oxidation of L-cysteine. *J. Electroanal. Chem.* **2010**, *638*, 241–245. [CrossRef]
24. Mardini Farias, P.A.; Rebello Wagener, A.; Altamirano Junqueira, A.; Castro, A.A. Determination of Cysteine in the Presence of Copper in Diluted Alkaline Electrolyte by Adsorptive Stripping Voltammetry at the Mercury Film Electrode. *Anal. Lett.* **2007**, *40*, 2032–2044. [CrossRef]

25. Yusoff, N.; Rameshkumar, P.; Noor, A.M.; Huang, N.M. Amperometric determination of L-cysteine using a glassy carbonelectrode modified with palladium nanoparticles grown on reduced graphene oxide in a Nafion matrix. *Microchim. Acta* **2018**, *185*, 246. [CrossRef]

26. Matsunaga, T.; Kondo, T.; Shitanda, I.; Hoshi, Y.; Itagaki, M.; Tojo, T.; Yuasa, M. Sensitive electrochemical detection of L-Cysteine at a screen-printed diamond electrode. *Carbon* **2021**, *173*, 395–402. [CrossRef]

27. Zhai, X.; Li, S.; Chen, X.; Hua, Y.; Wang, H. Coating silver metal-organic frameworks onto nitrogen-doped porous carbons for the electrochemical sensing of cysteine. *Microchim. Acta* **2020**, *187*, 493. [CrossRef]

28. Jerome, R.; Keerthivasan, P.V.; Murugan, N.; Devi, N.R.; Sundramoorthy, A.K. Preparation of Stable CuO/Boron Nitride Nanocomposite Modified Electrode for Selective Electrochemical Detection of L-Cysteine. *Chem. Sel.* **2020**, *5*, 9111–9118. [CrossRef]

29. Mazloum-Ardakani, M.; Alizadeh, Z. A Certain Electrochemical Nanosensor Based on Functionalized Multi-Walled Carbon Nanotube for Determination of Cysteine in the Presence of Paracetamol. *J. Nanostruct.* **2020**, *10*, 258–267. [CrossRef]

30. Manibalan, G.; Murugadoss, G.; Thangamuthu, R.; Kumar, M.R.; Kumar, R.M.; Jayavel, R. CeO_2-based heterostructure nanocomposite for electrochemical determination of L-cysteine biomolecule. *Inorg. Chem. Commun.* **2020**, *113*, 107793. [CrossRef]

31. Manibalan, G.; Murugadoss, G.; Thangamuthu, R.; Kumar, M.R.; Kumar, R.M. Facile synthesis of CeO_2-SnO_2 nanocomposite for electrochemical determination of L-cysteine. *J. Alloys Compd.* **2019**, *792*, 1150–1161. [CrossRef]

32. Atacan, K. $CuFe_2O_4$/reduced graphene oxide nanocomposite decorated with gold nanoparticles as a new electrochemical sensor material for L-cysteine detection. *J. Alloys Compd.* **2019**, *791*, 391–401. [CrossRef]

33. Beitollahi, H.; Ganjali, M.R.; Norouzi, P.; Movlaee, K.; Hosseinzadeh, R.; Tajik, S. A novel electrochemical sensor based on graphene nanosheets and ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl) acetate for electrocatalytic oxidation of cysteine and tyrosine. Meas. *J. Int. Meas. Confed.* **2020**, *152*, 107302. [CrossRef]

34. Li, Z.; Xu, H.; Wu, D.; Zhang, J.; Liu, X.; Gao, S.; Kong, Y. Electrochemical Chiral Recognition of Tryptophan Isomers Based on Nonionic Surfactant-Assisted Molecular Imprinting Sol-Gel Silica. *ACS Appl. Mater. Interfaces* **2019**, *11*, 2840–2848. [CrossRef]

35. Kurniawan, A.; Kurniawan, F.; Gunawan, F.; Chou, S.H.; Wang, M.J. Disposable electrochemical sensor based on copper-electrodeposited screen-printed gold electrode and its application in sensing L-Cysteine. *Electrochim. Acta* **2019**, *293*, 318–327. [CrossRef]

36. Zhou, H.; Ran, G.; Masson, J.F.; Wang, C.; Zhao, Y.; Song, Q. Rational Design of Magnetic Micronanoelectrodes for Recognition and Ultrasensitive Quantification of Cysteine Enantiomers. *Anal. Chem.* **2018**, *90*, 3374–3381. [CrossRef]

37. Premlatha, S.; Selvarani, K.; Bapu, G.N.K.R. Facile Electrodeposition of Hierarchical Co-Gd_2O_3 Nanocomposites for Highly Selective and Sensitive Electrochemical Sensing of L-Cysteine. *Chem. Sel.* **2018**, *3*, 2665–2674. [CrossRef]

38. Pei, L.Z.; Wei, T.; Lin, N.; Zhang, H.; Fan, C.G. Bismuth Tellurate Nanospheres and Electrochemical Behaviors of L-Cysteine at the Nanospheres Modified Electrode. *Russ. J. Electrochem.* **2018**, *54*, 84–91. [CrossRef]

39. Buljac, M.; Krivić, D.; Škugor Rončević, I.; Vladislavić, N.; Vukadin, J.; Buzuk, M. Voltammetric behaviour and amperometric sensing of hydrogen peroxide on a carbon paste electrode modified with ternary silver-copper sulfides containing intrinsic silver. *Monatsh. Chem.* **2020**, *151*, 511–524. [CrossRef]

40. Brinić, S.; Buzuk, M.; Bralić, M.; Buljac, M.; Jozić, D. Cu (II) Ion-Selective Electrode Based on Mixed Silver-Copper Sulfide: Phase Structure and Electrochemical Properties. *Int. J. Electrochem. Sci.* **2012**, *7*, 5217–5230.

41. Andersson, L.O. Study of Some SilverThiol Complexes and Polymers: Stoichiometry and Optical Effects. *J. Polym. Sci. Part A1 Polym. Chem.* **1972**, *10*, 1963–1973. [CrossRef]

42. *Report No. ISO 11843-2*; Capability of Detection. International Organization for Standardization (ISO): Genève, Switzerland, 2000.

43. Buglak, A.A.; Ramazanov, R.R.; Kononov, A.I. Silver cluster–amino acid interactions: Quantum-chemical study. *Amino Acids* **2019**, *51*, 855–864. [CrossRef]

44. Arenas, J.F.; Castro, J.L.; Otero, J.C.; Marcos, J.I. Study of Interaction Between Aspartic Acid and Silver by Surface-Enhanced Raman Scattering on H_2O and D_2O Sols. *Biopolymers (Biospectroscopy)* **2001**, *62*, 241–248. [CrossRef]

45. Han, C.; Xu, K.; Liu, Q.; Liu, X.; Li, J. Colorimetric sensing of cysteine using label-free silver nanoparticles. *Sens. Actuators B Chem.* **2014**, *202*, 574–582. [CrossRef]

46. Borase, H.P.; Patil, C.D.; Salunkhe, R.B.; Suryawanshi, R.K.; Patil, S.V. Bio-Functionalized Silver Nanoparticles: A Novel Colorimetric Probe for Cysteine Detection. *Appl. Biochem. Biotechnol.* **2015**, *175*, 3479–3493. [CrossRef]