J. Electrochem. Sci. Eng. **12(6)** (2022) 1081-1098; http://dx.doi.org/10.5599/jese.1561

Open Access : : ISSN 1847-9286
www.jESE-online.org

Review paper

An overview of recent advances in the detection of ascorbic acid by electrochemical techniques

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Received: October 24, 2022; Accepted: November 7, 2022; Published: November 30, 2022

Abstract

Ascorbic acid is a water-soluble vitamin essential in human nutrition, an antioxidant, a scavenger of free radicals in biological systems, and a cofactor of several enzymes. The reference range for ascorbic acid in healthy people is 6 - 20 mg L^{-1}. The variable concentration of ascorbic acid within biology fluids was found in clinical investigations to be a metric for assessing the exact amount of oxidative stress in the body's metabolism. Electroanalytical techniques are a group of methods in analytical chemistry, especially with extensive application in pharmaceutical industries. These techniques attracted further attention due to their unique characteristics, such as reduced sample or solvent consumption, high analysis speed, low operating cost, and high sensitivity, which made them suitable candidates for replacement or supplementation for spectrophotometry and separation approaches. The purpose of this article is to scrutinize the mechanisms and applications of current electroanalytical methods, including amperometric techniques, square wave voltammetry, differential pulse voltammetry and cyclic voltammetry, in their applications in pharmaceutical analysis for the detection of ascorbic acid. Related examples have been cited in the form of selected studies.

Keywords

Electrochemical sensors; cyclic voltammetry; modified electrode; differential pulse voltammetry

Introduction

Vitamin C (Vit C) or L-ascorbic acid (AA) is a ubiquitous water-soluble antioxidant found in numerous foodstuffs and biosystems. The physiologically and biochemically active Vit C is an L-enantiomer of ascorbic acid with γ-lactone structure, which is a pivotal factor in the formation of collagen as a key structural protein in the various body tissues. Evidence shows the direct role of AA in many bioprocesses, including neuropeptide amidation, synthesis of adrenal cortical hormones,

wound healing, amino acid metabolism, iron adsorption, free-radical or singlet oxygen scavenging and chelation. This potent antioxidant is a donor of two electrons by transferring a hydrogen atom to form the ascorbate radical ion, called semidehydroascorbic acid and dehydroscorbic acid. AA also reportedly conserves oxidizable components such as flavor and phenolic compounds. AA can develop relatively unreactive radicals that are difficult to amplify because it reacts rapidly with hydroxyl radicals. AA can control diseases caused by free radicals due to its strong antioxidant activity. Hence, the level of AA in the body is regulated through precise and programmed mechanisms [1-3]. The allowable level of AA in the body is estimated at 0.6 to 2 mg/dL. Any change in the level of this compound directly leads to the development of many diseases [4]. For example, AA deficiency results in conditions such as cardiovascular disease (CVD), Parkinson's disease (PD), Alzheimer's disease (AD), scurvy, rheumatoid arthritis and even cancer [5-7], as well as a higher level of AA is associated with gastric irritation, diarrhea and urinary stones. High concentrations of AA in the body make it a powerful antioxidant only in aqueous solutions and in the absence of heavy metal cations because the heavy metal cations force it to play the role of a pro-oxidant that either forms reactive oxygen species (ROS) or blocks the antioxidant activity, resulting in the induction of oxidative stress. Accordingly, it can be claimed that it is vital to detect the presence of AA in biological fluids to diagnose multiple disorders, which can help its appropriate application in the food industry, pharmaceutical formulations and cosmetics [8-11]. Therefore, the detection of AA is one of the fields of interest of research considering the importance of AA in industrial applications and human life.

There are currently diverse techniques to detect the AA, including potassium iodate [12], fluorimetry [13], ultraviolet-visible spectroscopy [14], reaction with hexacyanoferrate(III) and oxidation with Cu(II)-neocuproine complex [16], chemiluminescence and high-performance liquid chromatography (HPLC) [18]. However, these techniques have some drawbacks, including the need for a pre-concentration step of the sample, prolonged duration of analysis, the need for skillful personnel, special equipment and expensiveness.

The electrochemical detection of AA is based on the fact that it is the most common biologically electroactive compound that is easily oxidized [19,20]. In comparison with conventional instrumental techniques, there are some unique advantages for the electrochemical methods, including high analysis speed, simplicity, potent selectivity and sensitivity, portability, relatively low operating cost and no need for sample pre-treatment [21-84].

Some problems with AA detection exist in physiological status, involving low AA concentration as well as interference with uric acid and dopamine in pharmaceuticals, food and biological media due to similar oxidation activities, thereby highlighting the necessity for the development of novel, easy and fast approach with great sensitivity and selectivity to detect the AA in various media [85,86].

To this end, an appropriate electrocatalyst can be modified on the bare electrode surface. For example, the direct electron transfer of analyte on the electrode surface can be achieved through chemically modified electrodes (CMEs) reportedly [87-100].

In the continuation of the discussion, it should be mentioned that nanomaterials currently act as common transducers for the improvement of electrode surface in terms of electro-conductive and electrocatalytic profiles, providing more rapid electron-transfer and analyte-transform at the sensing interface. The nanomaterials, with their wide specific surface area (SSA) cause an interaction with dissolved materials. Accordingly, the precise selection of nanomaterials is the basis for the development of highly selective and sensitive supramolecular equipment with affinity to the analyte of interest [101-114]. Recent biological and chemical sensing electrodes have benefited from core/shell nanoparticles [115-118], metal nanostructures [119-126], oxides [127-135], bimetallic

compounds [136,137], carbon-based structures [138-140] and other mediators [141,142]. Chemical modification is possible for all electrodes used in electrochemical measurements, including carbon paste electrode gold electrodes, screen-printed electrodes and glassy carbon electrodes [143-145]. The purpose of this review article was to scrutinize different electrochemical methods previously used for AA detection, as well as recent developmental advances in different interface materials to modify the electrodes used in these methods.

Electrochemical methods to detect ascorbic acid

Electrochemical approaches refer to the transfer of charge between the electrode surface and the solid or liquid phase. This charge transfer procedure is amplified by electrical conductivity and interfacial reactions in the main solution. These are user-friendly methods because of no need for reagents, simplicity and cost-effectiveness, which can be used for in situ measurements through miniaturization and automation. Other advantages of such methods are the need for the least changes of samples, no reagent-caused contamination, or minimal loss by adsorption on containers compared to other analytical methods [146-150]. Scientists have recently made advances in improving sensitivity and limit of detection (LOD), so there are now several electrochemical methods with current or potential modulation, including amperometric techniques, square wave voltammetry, differential pulse voltammetry and cyclic voltammetry. In addition, surface functionalization enhanced selectivity, especially for AA detection.

Cyclic voltammetry method to detect ascorbic acid

In a study by Tashkhourian *et al.* [151], MOF MIL-101(Cr) was fabricated hydrothermally with a large pore volume, which played a role as an electrocatalyst in electrochemical processes for AA detection. To determine the electrocatalytic process of a MIL-101(Cr)-modified carbon paste electrode, the CV method was used to evaluate the electrooxidation of AA. Empirically optimized electrode exhibited a linear relationship of oxidation peak current with AA levels (0.01 to 10.0 mM) having the limit of detection (LOD) of 0.006 mM ($3S_b/m$). The method was tested to detect AA levels in two real samples of vitamin C effervescent tablet and vitamin C tablet, which showed a recovery rate of 96.0 and 97.0 %, respectively [151].

Chu *et al.* [152] replaced the enzymes with platinum nanoparticles (Pt NPs) as a novel non-enzymatic sensor electrocatalyst for AA detection. The graphene modified by poly(dimethyl diallyl ammonium chloride) (PDDA) elevated the modification capacity of nanomaterials as well as provided great solubility and conductivity. A potent catalytic impact on AA was reported for the Pt NPs. The integration of both materials and the subsequent modification on glassy carbon electrodes (GCE) led to the synthesis of a non-enzymatic sensor in accordance with a nanocomposite of a PDDA-functionalized reduced graphene oxide-Pt NPs (PDDA-RGO/Pt NPs/GCE). In their study, the CV method was employed to evaluate the electrochemical behavior of PDDA-RGO/Pt NPs, the results of which confirmed the capacity of the sensor for electrocatalytic AA detection with a linear range of 0.001 to 10.0 mM at 0 V and a low LOD of 0.0005 µM (S/N=3) [152].

Ahmed *et al.* [153] electrodeposited zinc oxide (ZnO) NPs from the aqueous solution of zinc nitrate, as electrochemical detectors, at 70 °C onto a glassy carbon electrode (ZnO/GCE). The CV method was applied to investigate the electrocatalytic oxidation of AA in the presence of 0.1 M phosphate buffer solution (PBS) at a pH value of 6.8. According to the response for AA oxidation on the bare electrode compared with the modified electrode, co-elevation in surface area and oxidation rate shifted the peak potential towards − 0.45 V on ZnO/GCE, having a greater transfer

coefficient and current density. The anodic peak current had a linear relationship with different AA levels (between 0.1 and 5.0 mM) [153].

Wu *et al.* [154] fabricated a porous g-C_3N_4(PCN)/Poly (3,4-ethylenedioxythiophene) composite-modified GCE (PCN/PEDOT/GCE) for the detection of AA using co-deposited strategy. They used CV to characterize the electrochemical properties of AA. The empirically optimal oxidation peak current was linearly related to different AA levels (between 10.0 and 1500.0 µM). A large electroactive area was obtained for the PCN/PEDOT composite with high electron transfer rate, making the composite a proper candidate for modified material to produce a sensor for the detection of AA [154].

The 3-chloropropyl silica gel (SG)-functionalized with imidazole ligand (SGI) was fabricated by da Silveira *et al.* [155]. Following the adsorption of cadmium ions, the SGI was combined with potassium hexacyanoferrate to produce CdHSGI, subsequently incorporated into a graphite paste electrode (CdHSGI/GPE) for the electrocatalytic detection of AA using CV method. The CV findings confirmed a redox couple with the mean potential of $E\theta'$=0.25 V (versus Ag/AgCl, $NaNO_3$ 1.0 mol L^{-1}; v = 20 mV s^{-1}), which was due to the process of $Fe^{2+}(CN)_6$/ $Fe^{3+}(CN)_6$. The voltammograms from the modified electrode for the AA detection showed a linear range between 0.10 and 0.90 mM (LOD = 0.79 mM) [231551].

Table 1 tabulates information about CV method based-electrochemical sensors, which have been reported by various works.

Table 1. CV method based-electrochemical sensors to detect AA

Electrochemical sensors	LOD, µM	Linear range, mM	Ref.
MIL-101(Cr)-modified carbon paste electrode	6.0	0.01 to 10.0	[151]
PDDA-RGO/Pt NPs/GCE	0.0005	0.001 to 10.0	[152]
ZnO/GCE	-	0.1 to 5.0	[153]
PCN/PEDOT/GCE	9.3	0.010 to 1.500	[154]
CdHSGI/GPE	0.79	10 to 90	[155]

DPV method to detect ascorbic acid

Murugan *et al.* [156] fabricated a catalytic material of mixed-phase 2D MXene for the co-detection of uric acid (UA), dopamine (DA) and AA biomolecules. They produced a Ti-C-Tx-modified GCE (Ti-C-Tx/GCE) sensor, whose electrochemical behavior was evaluated by DPV and CV, the results of which displayed potent electrocatalytic properties and individual oxidation peaks at 0.33, 0.2 and 0.01 V for UA, DA and AA, respectively. The synthesized sensor could co-detect the biomolecules in physiological pH values of 0.5 - 4.0 µM and 100.0 - 1500.0 µM for UA, 5.0- 50.0 µM for DA and 100.0-1000.0 µM for AA, with the LOD of 0.075, 0.06 and 4.6 µM for UA, DA and AA, respectively [156].

MoS_2/acid-exposed multi-walled carbon nanotubes (MWCNTs) composite (Ms-atCNTs) was first produced by Kumar *et al.* [157]. Subsequently, they applied this composite for the modification of the carbon paste electrode (CPE) and electropolymerized the alanine using NaOH. The modified electrode (p-Aln/Ms-atCNTCPE) produced was analyzed by CV and DPV methods to co-detect guanine (GU), serotonin (5-HT), AA and DA in PBS. The GU, 5-HT, AA and DA were co-detected electrochemically by the p-Aln/Ms-atCNTCPE. Moreover, the variation in concentration of the biomolecules was also cleared, with the LOD of 0.1, 0.1, 3.9 and 0.08 µM for GU, 5-HT, AA and DA, respectively [157].

In a study, a screen-printed carbon electrode covalently modified with self-assembled gold-decorated-polydopamine nanospheres (Au-PDNs/SPCE) as a novel sensor was fabricated by Arroquia *et al.* [158]. The sensor was utilized to co-detect the biomolecules of tryptophan (TR), UA,

DA and AA. The Au-PDNs were loaded on Au-NPs electrodeposited onto bare electrodes using cysteamine-glutaraldehyde bridges (Figure 1). The novel tool responded pH-dependently to these analytes, choosing the optimal working conditions as a function of features for the sample. The co-detection of TR, UA and AA in exposed to DA, and TR, UA and DA in exposed to AA was possible at pH of 3.0 and 8.0, having high sensitivity and broad linear range. TR, UA, DA and AA were co-detected at the pH value of 6.0 with competitive sensitivities in two consecutive linear ranges of 1.0 to 160.0 and 160.0 to 280.0 µM; 10.0 to 120.0 and 120.0 to 350.0 µM; 1.0 to 160.0 and 160.0 to 350.0 µM and 10.0 to 80.0 and 80.0 to 240.0 µM, with the LOD of 0.2, 0.1, 0.1 and 0.1 nM, respectively [158].

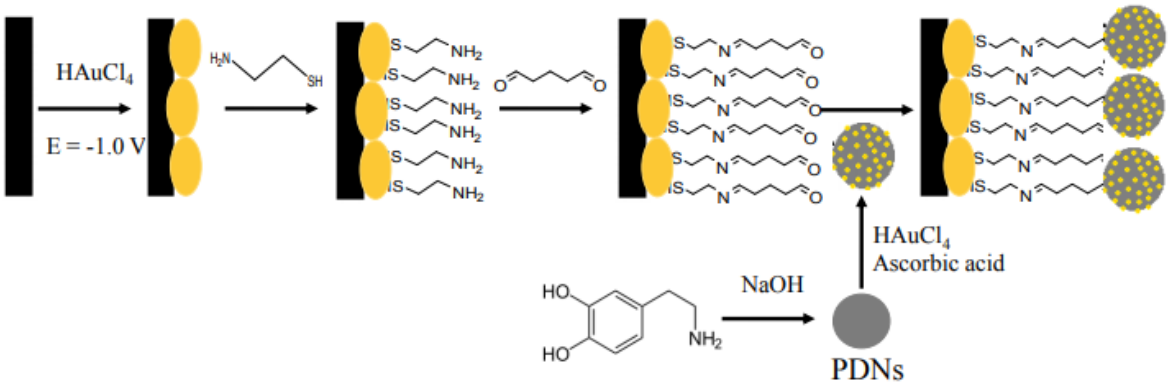

***Figure 1**. Schematic process of electrode preparation [158]*

Shalini *et al.* [159] applied a cellulose template for modification of polypyrrole grafted cellulose (PPy@C) surface through in-situ oxidation polymerization using ammonium peroxydisulfide (APS) as an oxidant under optimal state. High sensitivity and stability were reported for the modified electrode of PPy@C/GCE during AA detection in the real sample of commercial fruits. The PPy@C/GCE exhibited an acceptable regression coefficient and low LOD (10.0 to 150.0 µM) in the optimal condition of DPV [159].

Li *et al.* [160] fabricated thin flakes of hexagonal boron nitride (h-BN) by a low-temperature combustion synthesis (LCS) method using nitric acid and carbothermal reduction. DPV and CV techniques were employed to evaluate electrochemically the glassy carbon electrode modified with flake BN (BN/GCE), the results of which introduced a new electrode with high electrocatalytic potential and potent selectivity for electrochemically AA, DA and UA detection, with the liner relationships between current intensities and concentrations of 30.0 to 1000.0; 0.5 to 150.0 and 1.0 to 300.0 M, and LOD of 3.77, 0.02 and 0.15 M, respectively [160].

In a study by Selvarajan *et al.* [161], the GCE modified with SnO_2/chitosan (SnO_2/CHIT/GCE) as a new nanocomposite to co-detect AA, DA, and UA using CV and DPV methods (Figure 2). The modified GCE showed high electrocatalytic performance compared to the bare electrode. The ternary mixture containing AA, DA and UA could be well separated from each other at a scan rate of 0.050 V with a potential difference of 0.168, 0.326 and 0.612 V in the CV, as well as 0.178, 0.337 and 0.592 V in the DPV between AA and DA, DA and UA, UA and AA respectively. According to DPV findings, a linear relationship was found between concentration and peak current at different concentrations of 1.0 to 100.0 µM for UA, 1.0 to 18.0 µM for DA and 20.0 to 220.0 µM for AA, with the LOD (*S*/*N*=3) of 0.89, 0.77 and 6.45 µM for UA, DA and AA, respectively [161].

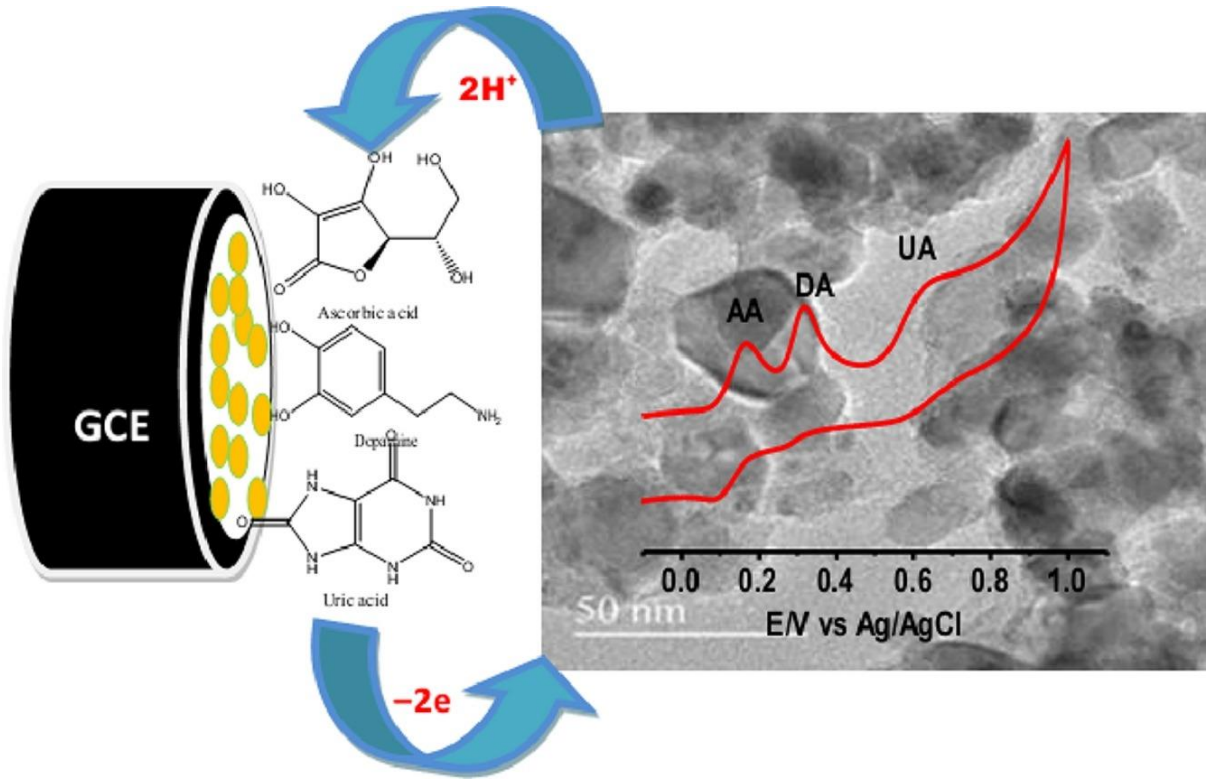

Figure 2. *Schematic process of glassy carbon electrode modified with SnO_2/chitosan (CHIT) nanocomposite to co-detect AA, DA and UA [161]*

Table 2 tabulates information about DPV method based-electrochemical sensors, which have been reported by various works.

Table 2. *DPV method based-electrochemical sensors to detect AA*

Electrochemical sensors	LOD, µM	Linear range, µM	Ref.
Ti-C-Tx/GCE	4.6	100.0 to 1000.0	[156]
p-Aln/Ms-atCNTCPE	3.9	22.0 to 200.0	[157]
Au-PDNs/SPCE	0.1 10^{-3}	1.0 to 350.0	[158]
PPy@C/GCE	0.75	10.0 to 150.0	[159]
BN/GCE	3.77 10^{6}	0.030 10^{-3} to 1.000 10^{-3}	[160]
SnO_2/CHIT/GCE	6.45	20.0 to 220.0	[161]

Square wave voltammetry method to detect ascorbic acid

In a study by Mohan *et al.* [162], bare toray paper was recruited as a working electrode whose electrocatalytic potential was analyzed by Ag/AgCl and platinum as a reference and counter electrodes, respectively. The oxidation of xanthine (X), DA, AA and UA via toray was tested by CV method. The sharpest oxidation peak appeared at certain non-interfering oxidation potentials. The CV was performed to test the principle of electron transfer at various scan rates, the results of which confirmed the presence of a surface-confined reaction. SWV and CV techniques in the absence of interference were applied to determine the electroactivity of these compounds. The obtained linear ranges included 10.0-1000.0 µM for UA, 7.0-300.0 µM for AA, 10.0-1000.0 µM for X and 30.0-1000.0 µM for DA, with the LOD values of 28.74 µM for UA, 97.12 µM for AA, 6.54 µM for X and 9.67 µM for DA [162].

In a study by Vedenyapina *et al.* [163], SWV and CV were carried out to analyze the electrochemical properties of AA on a boron-doped diamond (BDD) electrode. They found that the voltammetric

response signal can be used to quantify AA content in the aqueous solution. A function of analytically direct correlation was estimated on BDD for SWVA, and the LOD was 1.87 µM for AA [163].

In a study, Ni NPs/poly (1,2-diaminoanthraquinone) modified GCE (Ni/PDAAQ@GCE) was constructed by Hassan *et al.* [164] as a highly sensitive sensor using CV. The Ni NPs were incurporated by anodic polarization. The produced Ni/PDAAQ@GCE was applied to co-detect UA, DA and AA using SWV. A strong electrocatalytic potential was found for the modified electrode relative to the electrooxidation of UA, DA and AA in single, binary and ternary complexes in 0.1 M NaOH solution. According to empirical data, the LOD values for UA, DA and AA were estimated at 1.2, 0.072 and 0.11 µM in a single complex and 0.12, 0.29 and 0.069 µM in a ternary complex, respectively [164].

Fu *et al.* [165] aimed to regulate the electrocatalytic potential of commercial graphene ink using a novel water immersion treatment, which can discard the additives present in graphene ink and thus clear the defects on the surface. A glass coated by graphene ink (G-30) was produced to co-detect the presence of UA, DA and AA using the CV method, the results of which exhibited the onset of electrocatalytic reaction following the penetration of additives within water immersing treatment. The optimized linear calibration curves for UA, DA and AA were estimated at 0.5 to 150.0, 3.0 to 140.0 and 50.0 to 1000.0 µM, with the LOD values of 0.29, 1.44 and 17.8 µM, respectively [165].

Maouche *et al.* [166] aimed to detect the AA by constructing a polyterthiophene (P3T)-modified AgNPs-doped platinum electrode (P3T/AgNPs-Pt electrode) as a new sensor. High sensitivity was seen for the AgNPs-doped P3T film in comparison with the films doped with other metallic particles (Pd, Au, Co and Cu). As well, the oxidation signals were enhanced in detection by SWV than by CV. Successful detection of AA can be achieved by optimizing multiple factors like film polymerization time and film immersion time in $AgNO_3$ solution, which were 20 minutes and 60 seconds, respectively. The LOD value estimated at S/N =3 was 0.517 nmol L^{-1} by SWV. The produced sensors exhibited a potent selectivity for AA detection, and the reusability was up to 6 times with the best recovery of about 95 % [166]. Table 3 tabulates information about SWV method based-electrochemical sensors, which have been reported by various works.

Table 3. *SWV method based-electrochemical sensors to detect AA*

Electrochemical sensors	LOD, µM	Linear range, µM	Ref.
Bare toray paper	97.12	7.0 to 300.0	[162]
boron-doped diamond electrode	1.87	20.0 to 200.0	[163]
Ni/PDAAQ@GCE	0.11	100.0 to 700.0	[164]
G-30	17.8	50.0 to 1000.0	[165]
P3T/AgNPs-Pt electrode	5.17 10^{-4}	-	[166]

Amperometric method to detect ascorbic acid

An analyte is detected using the amperometric method through the measurement of the current at a constant applied potential of the working electrode relative to the reference electrode. The potential in this method is directly stepped to the value of interest, followed by measuring current. The amperometric sensors provide extra selectivity due to redox potential in the analysis of analyte species [167].

In the study by Xiao *et al.* [168], a new analytical approach with high simplicity, rapidity and stability was introduced to detect AA with the aid of a non-enzymatic amperometric sensor of GCE loaded by mesoporous $CuCo_2O_4$ rods ($CuCo_2O_4$/ GCE). Figure 3 presents a mechanism of electrocatalytic oxidation of the electrode modified with porous $CuCo_2O_4$ for the detection of AA. The produced structure acted as an excellent electrocatalyst to detect AA according to plotted *I-t* curve.

The synthesized sensor sensitivity was estimated at 9.482mA $mM^{-1}cm^{-2}$ at different AA concentrations between 1.0 and 100.0 mM and 2.474 mA mM^{-1} cm^{-2} at AA concentrations between 100.0 and 1000.0 mM. The LOD was decreased to 0.21 mM (R^2= 0.99) [168].

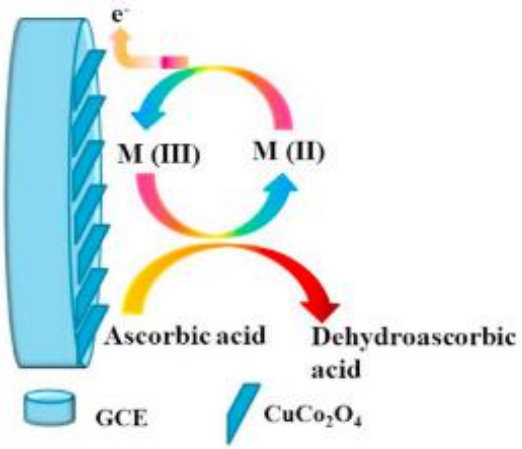

Figure 3. *The possible pathway of electrocatalytic oxidation of electrodes modified with porous $CuCo_2O_4$ for detection of AA [168]*

One of the simple approaches to developing electrochemical sensors is the optimization of the electrocatalytic behavior of porous metallic structures with large surface areas (such as nanoporous gold, NPG) through structural manipulation. For example, a selective, sensitive and robust electro-analytical structure was developed by Kumar *et al.* [169] based on NPG to detect AA present in acidic extracts of Arabidopsis thaliana and Aspergillus. To this end, potentiostatic electrodeposition was used to electrodeposit the NPG films on a gold microelectrode (NPG-modified gold microelectrodes). Amperometric parameters were measured at 0.3 V *vs.* Ag/AgCl (sat. KCl) in the acidic electrolyte to detect AA in biological samples and minimize autoxidation. Modification of usual microelectrodes with NPG film increased the sensitivity by about 1000-fold, which reached 2.0 nA μmol^{-1} L^{-1}. The LOD of AA was calculated on the basis of a calibration curve of 2.0-µM flow concentration [169].

In a study by Yu *et al.* [170], molybdenum oxide (MoO_x) plus Prussian blue (PB) were loaded on graphite felt (GF) as a sensor (MoO_x@PB/GF) for AA detection. MoO_2 nanorods produced by Mo-precursor annealing enhance the PB fabrication from $[Fe(CN)_6]^{3-}$ and Fe^{3+}. An excellent electro-catalytic oxidation was seen for AA by the modified electrode with a high sensitivity of 0.37 A/M, a strong selectivity, significant reproducibility, an acceptable linear response at concentrations of 0.0125-293.0 mM, and a very low LOD of 0.0119 mM (*S*/*N*= 3) [170].

In a study by Hei *et al.* [171], 3D hierarchical N-doped nano-scaled carbons (3D-NNCsHAs) were produced with unique properties, including eco-friendly, cost-effectiveness, great precursor, large surface area, abundant defective sites, and wide distribution of pore size by sea-tangle (*Laminaria japonica*) pyrolysis. As seen in Figure 4, the produced 3D-NNCsHAs were applied to fabricate a sensor for AA detection with high selectivity and sensitivity. The 3D-NNCsHAs-modified GCE (3D-NNCsHAs/GCE), when comparing with GCE and carbon nanotubes-modified GCE (CNTs/GCE), could better electrocatalytically detect AA, with smaller LOD of 1.0 µM, broader linear range between 10.0 and 4410.0 µM and shorter electrooxidation peak potential (-0.02 V *vs.* Ag/AgCl). Moreover, greater anti-fouling and anti-interference activities were reported for AA detection by the 3D-NNCsHAs/GCE [171].

Figure 4. *Schematic process of inexpensive production of an amperometric sensor of nitrogen-doped nanocarbons using sea-tangle to detect ascorbic acid [171]*

Scremin *et al.* [172] introduced TiO_2-Au NP integrated with MWCNT- modified GCE (TiO_2-Au NP-MWCNT-DHP/GCE) in a dihexadecyl phosphate film for AA detection, the results of which indicated rapid charge transfer and irreversible anodic property using CV. The analytical curve in optimized conditions and amperometry of 0.4 V exhibited linear AA concentration between 5.0 and 51.0 µmol L^{-1}, having LOD of 1.2 µmol/l [172].

The production of an organic electrochemical transistor sensor (OECT) was reported by Zhang *et al.* [173] using a gate electrode modified with molecularly imprinted polymer (MIP) film for AA detection. A strongly selective and sensitive OECT sensor was built by integrating the OECT amplification function and MIP selectivity. The formula of the MIP film-modified OECT sensor is shown in Figure 5A, and the principle of action of the MIP film-modified electrode is seen in Figure 5B. All stages of producing modified electrodes and the adsorption ability of MIP/Au electrodes were analyzed by the CV method and electrochemical impedance spectroscopy (EIS). A sensitivity of 75.3 µA channel current change per decade and a small LOD of 10.0 nM ($S/N > 3$) were reported for the MIP-OECT sensor after altering relative AA concentration from 1.0 to 100.0 µM under optimal conditions [173].

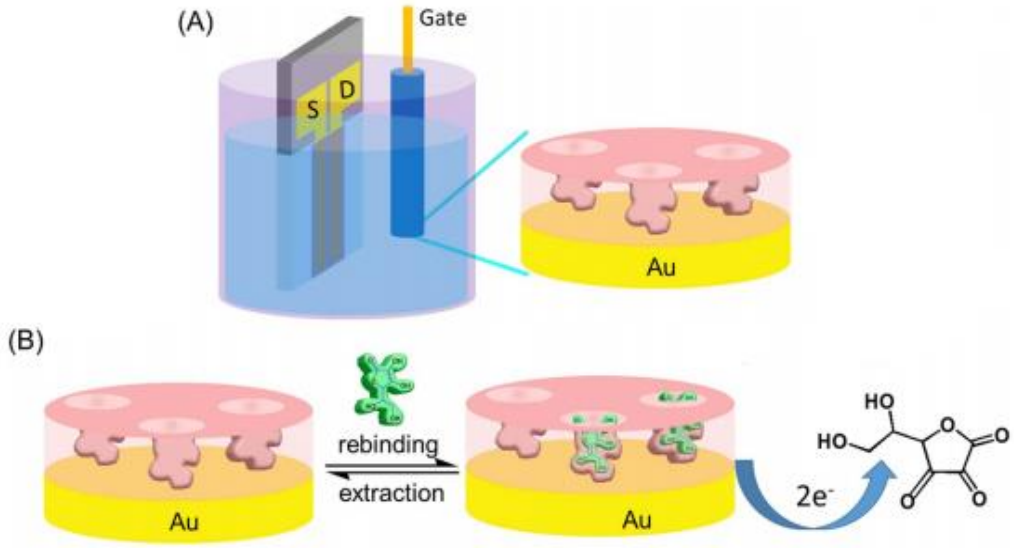

Figure 5. *(A) Schematic process of MIP-modified OECT sensor for AA detection. (B) Schematic process of AA removing and rebinding on surface of MIP film and of AA oxidation on surface of modified gate electrode [173]*

Table 4 tabulates information about *amperometric* method based-electrochemical sensors, which have been reported by various works.

Table 4. *Amperometric method based-electrochemical sensors to detect AA*

Electrochemical sensors	LOD, µM	Linear range, mM	Ref.
$CuCo_2O_4$/ GCE	210 mM	1.0 to 1000.0	[168]
NPG modified gold microelectrodes	2.0	0.010 to 1.100	[169]
MoO_x@PB/GF	11.9	0.0125 to 293.0	[170]
3D-NNCsHAs/GCE	1.0	0.010 to 4.410	[171]
TiO_2-Au NP-MWCNT-DHP/GCE	1.2	0.005 to 0.051	[172]
MIP-OECT	0.010	0.001 to 0.100	[173]

Conclusion

In recent years, biomedical and pharmaceutical investigations and procedures have mainly focused on diverse electrochemical techniques based on voltammetric (SWV, DPV and CV) and amperometric methods to a lesser extent. The need for near-patient testing or in vivo real-time analysis significantly enhances the functional parameters of the electrochemical method. There is still a need for further studies to increase the sensitivity and selectivity of the desired techniques. Accordingly, a variety of new materials and surface modification methods have been proposed so far to fabricate the diagnostic electrodes for the detection of ascorbic acid, including screen-printed electrodes, carbon-based electrodes, nanoparticles, application of carbon-based nanotubes, enzymes, graphene, mediators and polymer films alone or in combination, to prevent the use of additives in the sample solution and minimize the sample preparation process. Preferred and highly applied methods in this field include screen-printed and miniaturized electrodes due to potent flexibility, cost-effectiveness, minimal sample concentration, high reproducibility for measurements, the ability of in vivo analysis and capacity for testing single biological cells. Although significant innovations have occurred in the construction or optimization of sensors, there are compromises and challenges on important functional items (such as biocompatibility, the limit of detection, sensitivity, selectivity and stability) with specific application goals (such as focused on matrix, type of analysis, sample concentration and ascorbic acid content). However, these electrochemical methods occupied a special place for analytical processes because of their unique advantages over more complex techniques (such as mass spectrometry and chromatography), including cost-effectiveness and no need for advanced equipment.

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