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Review

Analytical Capabilities of Coulometric Sensor Systems in the Antioxidants Analysis

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Abstract: The definition of antioxidants (AOs), their classification and properties as well as electrochemical sensor systems for AOs analysis are briefly discussed. The analytical capabilities of coulometric titration with electrogenerated titrants as sensor systems for AOs determination have been considered in detail. The attention focused on the individual AO quantification that was mainly used in the pharmaceutical analysis and estimation of total antioxidant parameters (total antioxidant capacity (TAC), ferric reducing power (FRP) and ceric reducing/antioxidant capacity (CRAC)) allowing the fast screening of the target samples including their quality control. The main advantages of coulometric sensor systems are pointed out. The selective quantification of individual AO in a complex matrix using a combination of chromatography with coulometric or coulometric array detection under potentiostatic mode is discussed. The future development of coulometric sensor systems for AOs analysis is focused on the application of novel coulometric titrants and the application of coulometric detection in flow injection analysis.

Keywords: coulometric titration; electrogenerated titrants; coulometric detection; antioxidants; antioxidant capacity; ferric reducing power; ceric reducing/antioxidant capacity

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1. Introduction

1.1. Antioxidants: Definition, Classification and Properties

Antioxidants (AOs) are among the attractive and important substances under investigation in the life sciences. The term "antioxidant" is widely used in the scientific literature. It has a different interpretation depending on the method used for the evaluation of antioxidant properties. In order to unify the terminology, Halliwell and Gutteridge considered an AO as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delay or prevent oxidation of that substrate" [1]. Later, the term was modified by the same authors as "any substance that delays, prevents or removes oxidative damage to a target molecule" [1]. This definition covers all oxidative and non-oxidative processes including the radical and non-radical type. The physiological role of AO is the prevention of cellular components' damage occurring from chemical reactions involving free radicals [2]. In general, the term "antioxidant" is not strict if the oxidizing agent neutralized by this AO is not taken into account [3]. Moreover, the applicability of the term depends on the conditions under which an AO acts, in particular, in vitro or in vivo. In this context, the detailed description of the reactions and conditions of the antioxidant effect evaluation becomes a basic factor. Outside of this context, the assignment substances to the AOs cannot give any biologically significant information [4].

The most common and effective AOs are tocopherols (vitamin E), carotenoids, ascorbic acid, wide range of natural and synthetic phenolic compounds (eugenol and its derivatives, gallic acid and its derivatives, hydroxybenzoic and hydroxycinnamic acids, flavonoids, stilbenes, substituted hydroxybenzenes), sulfur-containing amino acids (cysteine, methionine, lipoic acid) and tripeptide glutathione, biogenic amines, some steroid hormones, phospholipids and blood proteins [5].

AOs can be classified into two large groups: proteins and low-molecular weight AO (Figure 1) [6,7]. Proteins are represented by a wide range of enzymes (superoxide dismutase, catalase, peroxidase, glutathione reductase, etc.) and non-enzymatic proteins (transferrin, albumin, ferritin, ceruloplasmin, etc.). The group of low-molecular weight AOs consists of a wide range of natural and synthetic compounds preventing the damage from the action of reactive oxygen and nitrogen species via direct or indirect interaction with them [8].

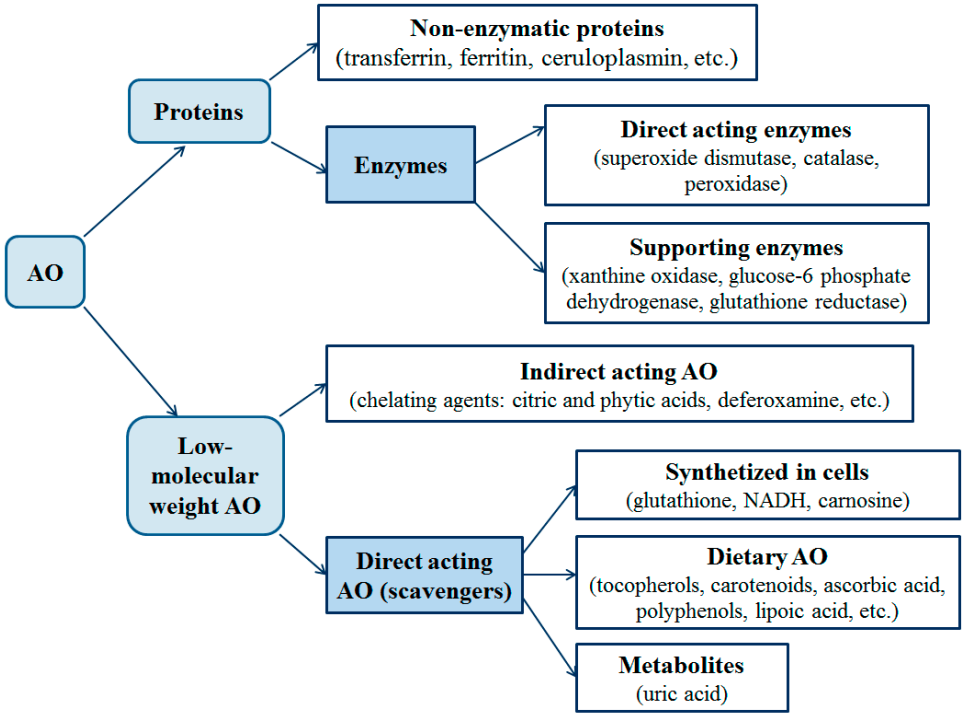

Figure 1. Classification of the antioxidants. Adapted from [6] with permission from Elsevier.

AO action can be classified in three ways [9]:

- Prevention of the formation of reactive oxygen and nitrogen species and their neutralization via subsequent reactions. Chelating agents, proteins and catalase act in such a way;
- Termination of cascade reactions of reactive oxygen and nitrogen species generation and scavenging of free radicals. This mechanism is used by low-molecular weight AOs, for example, α-tocopherol, ascorbic acid, polyphenols;
- Restoration of damage to macromolecules caused by exposure to reactive oxygen species and their derivatives with the help of enzymatic systems.

According to the source, AOs can be considered as endogenous (synthesized in the body) and exogenous (coming from the environment). The latter can undergo further transformation in the human body, giving other forms of AOs. This type of AO can be considered as partially endogenous. The main exogenous AO sources for humans are foodstuff and pharmaceuticals. The low-molecular weight AOs of exogenous origin acting as radical scavengers represented by the largest number of organic compounds of various natures are the most studied ones. For example, the number of natural phenolic AOs (secondary metabolites from fruits, vegetables, plants, etc.) reached more than ten thousand [10]. The high-rate constant of the reaction between low-molecular weight AOs and reactive oxygen and nitrogen species is usually obtained [9]. The protective effect of these AOs is based on the formation of intermediate radicals and molecules that are significantly less active than the initial radicals [1,9].

There is equilibrium between oxidants and AOs in living systems that is regulated within the framework of homeostasis. The balance support involves therapeutic correction and the monitoring of the processes mentioned above. In this case, the concentration of AO applied should be controlled as far as low-molecular weight AOs can exhibit prooxidant activity that depends on the concentration, structure and mechanism of AO action. The major role in the prooxidant effect plays a concentration of low-molecular weight AO. Outside the therapeutic dose, toxic and prooxidant properties can prevail, for example, the ability to induce apoptosis of healthy cells [11].

On the other hand, the prooxidant properties of AOs cannot be considered as purely toxic since they are associated with signaling cellular systems. The toxic effect of AOs occurred only at high concentrations in vivo. In addition, the prooxidant effect may have a positive effect by inducing low-intensity oxidative stress. This leads to the activation of the antioxidant defense system of cells, and the biotransformation of some enzymes providing a cytoprotective effect [12].

AOs, as mentioned above, are one of the components of foodstuff. The origin or nature of the food, the presence of macrocomponents as well as the biochemical processes occurring in a living organism significantly affect the AO properties. Thus, the bioavailability of AOs, their chemical form, reactivity and transformations should be taken into account for the correct estimation of AOs biological activity. This problem is especially actual for the phenolic AOs which bioavailability and bioaccessibility changes in a wide range due to the matrix effects (interaction with lipids, polysaccharides and proteins [13–15] contained in food). The AOs bioavailability and bioaccessibility have been discussed in details in the reviews [15–20].

Summarizing the aspects mentioned above, the rapid and simple methods for the evaluation of AOs contents in their sources as well as biological systems are required.

1.2. Electrochemical Sensor Systems for Antioxidants Analysis

Reactions of AO with free radicals based on the electron transfer allow the application of the electroanalytical methods for their determination. High sensitivity, the rapidity of the procedure and relatively low costs, in addition to possibility of miniaturization make electroanalytical methods very attractive tools for the antioxidant analysis [7,15,21–25] and an alternative to chromatography in screening application. Moreover, these methods can be combined with the detection in different types of liquid chromatography and capillary electrophoresis.

The reactivity of AO is shown in two types of processes. The first group of approaches is based on the AO oxidation at the electrode surface under conditions of voltammetry [7,21,25], chronoamperometry [26–33] or chronocoulometry [34,35]. The majority of these methods include the application of chemically modified electrodes providing high sensitivity and improving the selectivity of the target AO response. The second group of electrochemical methods is focused on the reactions of AO in the solution with the oxidants. This type of interaction can be realized via the reaction with the redox probe introduced into the solution with further potentiometric detection [21,36]. Another method for the reaction is interaction with the oxidant that is electrochemically generated on the electrode surface under constant-current or constant-potential coulometry conditions [7,37–39]. These reagents are called coulometric titrants.

Thus, a wide range of electrochemical sensors and electrochemical sensor systems for AOs have been successfully developed. The current review is focused on the analytical capabilities of the different modes of coulometry as sensor systems applied in the AOs analysis.

2. Coulometric Sensor Systems

Coulometry is still the only physicochemical method that does not use the dependence of the analytical signal on the concentration of the analyte, i.e., coulometry is an absolute method similar to gravimetry and does not require chemical standards or calibration. Since

it is based on Faraday's law, the mass of the analyte is proportional to the quantity of electricity consumed for its electro-oxidation/electroreduction. The electrical charge can be measured with high accuracy and precision. Thus, the use of coulometry decreases the error and increases the accuracy of the results obtained, as well as significantly reduces the analysis time (in comparison with classical methods) [40]. Nevertheless, insufficient attention is paid to coulometry that is almost out of practical applications in industry and research laboratories. The most used coulometric approach is that quantification of water in different samples using Karl Fischer titration [41].

There are two options of coulometric determination: the constant-current and the constant-potential modes. The former is inherently simpler and successfully used in the AOs analysis under conditions of coulometric titration. In this case, coulometric titration is based on the electrochemical generation of the suitable titrant under constant-current conditions. This is also a very attractive form of providing reagents for systems based on the in situ generation by electrolysis and further reactions with the AO being determined. Therefore, in fact, the electrons play the role of the titrant. In other words, the method is based on true titration where the sample volume and the amount of reagent added up to the end point are the only parameters necessary for the AOs quantification.

The main advantages of constant-current coulometry with electrogenerated titrants are:

- No necessity to use standard solutions and a calibration graph (the method is absolute);
- The absence of a titrant standardization step as far as the quantity of the electrogenerated titrant is determined by the quantity of electricity;
- The possibility to use unstable reagents for which standard solutions cannot be prepared due to their high reactivity;
- The absence of solution dilution during titration;
- Small volume of the sample;
- Microamounts of analyte can be quantified;
- The possibility of the quantification of electrochemically inert compounds by a chemical reaction with the titrant;
- High accuracy and reproducibility of the determination;
- Easiness of automation;
- Rapidity (the titration time does not exceed 5 min);
- Simplicity;
- Wide range of analytes and samples to be studied.

Spectrophotometric, chemiluminescent, potentiometric and amperometric methods are used for the coulometric titration end point indication [42]. The most common method is amperometry with two polarized electrodes, or the so-called biamperometry (dead-stop end point).

The simplicity and affordability as well as the possibility of miniaturization and automation give an opportunity for the wider usage of the method in the laboratory practice.

3. Constant-Current Coulometry with Electrogenerated Titrants in Antioxidants Analysis

Taking into account the nature of AOs, electrogenerated titrants acting as oxidizing agents (titrants–oxidants), in particular halogens (chlorine, bromine, and iodine), hexacyanoferrate(III) and Ce(IV) ions are used. The titrants are generated in constant-current (galvanostatic) mode. The amount of titrant obtained and its reaction with AOs are controlled using biamperometric detection.

3.1. Electrogeneration of Titrants–Oxidants and Their Properties

The electrogeneration of the titrants under galvanostatic conditions with biamperometric detection is usually performed in four-electrode cells consisting of two electrochemical circuits (the generating and indicator ones) (Figure 2). The electrodes should be fabricated from the inert materials. The most often platinum is used for this purpose [43]. The working electrode on which the electrogeneration of the titrant occurs should have a high surface

area (platinum plate electrode is one of the typical). The counter electrode has to be isolated from the electrolyte with a semi-permeable membrane in order to avoid side reactions like the reaction of the main product at the counter electrode with analyte or titrant, the reaction of electrogenerated titrant on the counter electrode or the reduction of the product of reaction between the analyte and titrant on the counter electrode with the formation of the initial form of analyte. The indicator circuit consists of two polarized platinum electrodes that are placed close to each other. The polarization potential of 200–300 mV is applied.

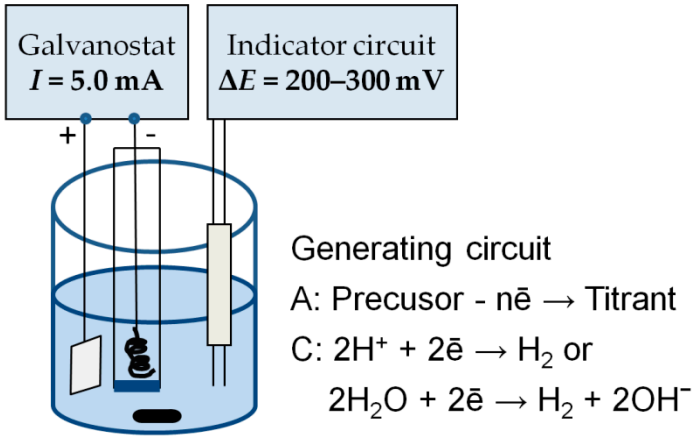

Figure 2. Scheme of the electrochemical cell for the coulometric titration.

The strict requirement of a coulometric titration is the 100% current efficiency of titrant generation that means the absence of side electrochemical reactions. Titrants–oxidants under consideration are generated on the anode via the electro-oxidation of the corresponding precursor in the suitable electrolyte. The current density of 5 mA cm^{-2} provides 100% of the current yield of the titrants [44]. The conditions and reactions of titrants–oxidants electrogeneration are summarized in Table 1.

Table 1. Conditions and reactions of the titrants–oxidants' electrogeneration for the coulometric titration of AO [44,45].

Titrant	Electrode	Precursor and Electrolyte	Anodic Process	Cathodic Process
Cl_2	Pt	0.2 M KCl in 0.1 M H_2SO_4	$2Cl^- - 2\bar{e} \rightarrow Cl_2$	$2H^+ + 2\bar{e} \rightarrow H_2$
	Pt	0.2 M HCl in 0.1 M $HClO_4$ in acetonitrile	$2Cl^- - 2\bar{e} \rightarrow Cl_2$	$2H^+ + 2\bar{e} \rightarrow H_2$
Br_2	Pt	0.2 M KBr in 0.1 M H_2SO_4	$2Br^- - 2\bar{e} \rightarrow Br_2$	$2H^+ + 2\bar{e} \rightarrow H_2$
	Pt	0.2 M $(C_2H_5)_4NBr$ in 0.1 M $HClO_4$ in acetonitrile	$2Br^- - 2\bar{e} \rightarrow Br_2$	$2H^+ + 2\bar{e} \rightarrow H_2$
I_2	Pt	0.1 M KI in acetate buffer pH 3.56	$2I^- - 2\bar{e} \rightarrow I_2$	$2H^+ + 2\bar{e} \rightarrow H_2$
$[Fe(CN)_6]^{3-}$	Pt	0.1 M $K_4Fe(CN)_6$ in 2 M KOH	$[Fe(CN)_6]^{4-} - \bar{e} \rightarrow [Fe(CN)_6]^{3-}$	$2H_2O + 2\bar{e} \rightarrow H_2 + 2OH^-$
Ce^{4+}	Pt	0.1 M $Ce(NO_3)_3$ in 3 M H_2SO_4	$Ce^{3+} - \bar{e} \rightarrow Ce^{4+}$	$2H^+ + 2\bar{e} \rightarrow H_2$

It should be noted that the electro-oxidation of bromide and iodide ions in acidic medium leads to the formation of tribromide (triiodide) anions, molecular bromine (iodine) and short-living bromine(iodine) radicals adsorbed on the generating electrode surface [44,46]. These species can take part in the radical and redox reactions as well as the reactions of the electrophilic substitution and addition to multiple bonds that provide the possibility to determine a wide range of AOs with different action.

The oxidants under consideration have different oxidative power. The strongest oxidants are electrogenerated chlorine and Ce(IV) while iodine and hexacyanoferrate(III) ions are the weak ones. These properties allow AO differentiation as well as the improvement of the AO quantification selectivity. On the other side, the application of one electron

titrants, in particular hexacyanoferrate(III) and Ce(IV), is of interest because this approach can exclude the side and competitive reactions affecting the AO determination and can simulate the reactions occurring in vivo.

3.2. Quantification of Individual Antioxidants

Electrogenerated coulometric titrants have been successfully applied for the quantification of a wide range of AO of different classes (water-soluble S-containing and phenolic compounds as well as lipophilic vitamins and water-insoluble polyphenols) (Table 2). The pharmaceutical dosage forms are usually studied as real samples. The auxiliary components of the samples do not react with the titrant and do not interfere with the determination of target AO.

Table 2. Coulometric sensing of individual antioxidants.

Antioxidant	Titrant	Number of Electrons	Sample	Ref.
S-containing AO				
Lipoic acid	Cl_2 Br_2 I_2	10 4 4	Tablets	[47]
Captopril	Cl_2 Br_2 I_2	6 6 2	Tablets	[48]
Sodium polydihydroxyphenylenethiosulfonate	Cl_2 Br_2	3 2	Pharmaceutical substance	[49]
Methionine	Cl_2 Br_2	4 2	Tablets	[50]
Glutathione	Cl_2 Br_2 I_2	6 6 2	Human blood	[51]
Liposoluble AO				
α-Tocopherol	Cl_2 Br_2 $[Fe(CN)_6]^{3-}$	2 2 1	Model solutions [2] Vegetable oil	[52] [53]
Retinol	Cl_2 Br_2	6 6	Model solutions	[52]
Natural Phenolic AO				
Rutin	Br_2 $[Fe(CN)_6]^{3-}$	4 4	Total flavonoids Pharmaceutical dosage forms	[54] [55]
Quercetin	$[Fe(CN)_6]^{3-}$	5	Pharmaceutical dosage forms	[55]
Dihydroquercetin	$[Fe(CN)_6]^{3-}$	5	Pharmaceutical dosage forms	[55]
Ellagic acid	Br_2 $[Fe(CN)_6]^{3-}$	4 4	Model solutions	[56]
Gallic acid	Br_2 $[Fe(CN)_6]^{3-}$	6 4	Model solutions	[56]
Syringaldehyde	Br_2 $[Fe(CN)_6]^{3-}$	2 0	Model solutions	[56]
Vanillin	Br_2 $[Fe(CN)_6]^{3-}$	2 0	Model solutions	[56]
Coniferaldehyde	Br_2 $[Fe(CN)_6]^{3-}$	5 0	Model solutions	[56]

Table 2. *Cont.*

Antioxidant	Titrant	Number of Electrons	Sample	Ref.
Sterically Hindered Synthetic Phenols				
Ionol (BHT [1])	Cl_2	2	Mineral oil	[57]
	$[Fe(CN)_6]^{3-}$	2	Vegetable oil	[53]
BHT amino derivatives	$[Fe(CN)_6]^{3-}$	2	Model solutions	[53]
Irganox® 1081	$[Fe(CN)_6]^{3-}$	2	Model solutions	[53]
Water-Soluble Synthetic Phenols				
Hydroquinone	Cl_2	2	Model solutions	[58]
	Br_2	2		
	$[Fe(CN)_6]^{3-}$	2		
Hydroquinone derivative	Cl_2	12	Model solutions	[58]
	Br_2	7		
	$[Fe(CN)_6]^{3-}$	4		
Catechol	Cl_2	2	Model solutions	[58]
	Br_2	2		
	$[Fe(CN)_6]^{3-}$	2		
Catechol derivatives	Cl_2	9–12	Model solutions	[58]
	Br_2	7		
	$[Fe(CN)_6]^{3-}$	4		
Pyrogallol	Cl_2	2	Model solutions	[58]
	Br_2	2		
	$[Fe(CN)_6]^{3-}$	2		
Pyrogallol derivatives	Cl_2	2–8	Model solutions	[58]
	Br_2	2–5		
	$[Fe(CN)_6]^{3-}$	2		
Others				
Ascorbic acid	I_2	2	Pharmaceutical dosage forms	[55]
			Vitamin C dietary supplement	[59]
			Dietary supplement tablets	[60]
	Br_2	2	Gelatin	[61]
	$[Fe(CN)_6]^{3-}$	2	Pharmaceutical dosage forms	[55]
Uric acid	Br_2	2	Model solutions	[62]

[1] BHT—butylated hydroxytoluene; [2] solution of AO in an appropriate solvent.

Taking into account the different strengths of the titrants–oxidants, there is the possibility of controlling the selectivity of the target analyte response. Thus, electrogenerated iodine does not oxidize phenolic AOs [55] and retinol [63], while it reacts with thiol-containing AOs [47–51] and ascorbic acid [55,59,60]. On the contrary, hexacyanoferrate(III) ions easily interact with phenolic AOs and ascorbic acid [55] but do not react with S-containing AOs.

The reaction products depend on the titrant used. For instance, thiol-containing compounds can be oxidized to disulfide, sulfoxide or sulfonic acid with the participation of two, four and six electrons, respectively [47–51]. Phenolic compounds are usually oxidized with the formation of *o*-quinone fragments [53,54,56–58]. α-Tocopherol is oxidized to *p*-tocopherylquinone by halogens [52] and to α-tocopheroxyl radical by hexacyanoferrate(III) ions [53]. In this case, the oxidation process is similar to that one occurring in living cells under influence of the reactive oxygen species [64].

The difference in reactivity of the titrants can be used in the analysis of relatively simple samples containing one or several AOs. A typical example is the determination of AOs as active principles in one–two-component pharmaceutical dosage forms [7]. For instance, the quantification of ascorbic acid and rutin or other polyphenols can be successfully realized

by coulometric titration with electrogenerated iodine being selective towards ascorbic acid and hexacyanoferrate(III) ions reacting with both AOs [55]. The difference in the quantity of electricity spent for the titration of the AO mixture and of ascorbic acid reflects the rutin/other phenolic AO contents in the sample.

Nevertheless, the relatively low selectivity of coulometric titration is one of the limitations of the method, particularly in the case of electrogenerated chlorine and bromine use. In some cases, the problem can be solved using preliminary extraction and derivatization. This approach has been successfully realized for the butylated hydroxytoluene extracted from the mineral oil prior to coulometric determination [57].

It should be noted that coulometric titration with electrogenerated halogens and hexacyanoferrate(III) ions can be realized in surfactant media [37]. The upper limits of surfactant content allowable to generate titrants with 100% current yield were determined. Low-molecular weight AOs (ascorbic acid, rutin, α-tocopherol and retinol) were determined by a reaction with the electrogenerated titrants in the surfactant media. Cationic surfactants can be used for the titration with halogens only. Anionic surfactant give good results in the case of hexacyanoferrate(III) ions. Nonionic surfactants can be applied with both types of titrants. The use of surfactants in coulometric titration of lipophilic AOs (α-tocopherol, retinol) provides their solubilization and allows preventing the application of organic solvents that significantly simplifies the determination, makes it cheaper as well as prevents the environment from toxic organic wastes that is in line with the "green chemistry" concept. The possible harmful effect of surfactants to the environment is overcome by the usage of their low concentrations as well as the application of biodegradable surfactants.

3.3. Evaluation of Total Antioxidant Parameters

The low selectivity of coulometric titrants can be successfully used for the evaluation of total contents of AOs or groups of AOs in the sample. In this case, the total antioxidant parameters can be used for the characterization of the sample as a whole. This allows to exclude the tedious and costly identification and quantification of individual AOs in the sample under investigation (the number of which can reach several hundreds). Furthermore, the total antioxidant parameters allow to take into account the possible synergistic or antagonistic effects of individual AOs and therefore predict the biological activity of the whole sample.

The application of coulometric sensor systems for these purposes is an attractive tool due to their simplicity, rapid procedure, high sensitivity and low cost. The choice of coulometric titrants gives the opportunity to control the type of AOs to be measured. Another advantage of the coulometric approaches is the use of an universal unit for the expression of the total antioxidant parameters, i.e., Coulombs as far as the quantity of electricity spent for the titration of the sample is considered as an analytical signal. Moreover, the total antioxidant parameters obtained can easily be recalculated in the equivalents of individual antioxidants using stoichiometric coefficients of their reaction with the corresponding titrants that simplify the comparison of the data with the ones for other methods.

The total antioxidant parameters to be evaluated using coulometric sensor systems are the total antioxidant capacity (TAC), ferric reducing power (FRP), and ceric reducing/antioxidant capacity (CRAC) based on the reactions of the AOs contained in the sample with coulometric titrants (the electrogenerated bromine, hexacyanoferrate(III) ions and Ce(IV), respectively). These approaches have been successfully applied to foodstuff, bioadditives and biomedical samples containing a "bouquet" of AO of different classes (Table 3). As one can see, the major AOs contributing to the total antioxidant parameters depend on the type of the sample and the titrant–oxidant applied.

Table 3. The total antioxidant parameters evaluated using coulometric sensor systems.

Antioxidant Parameter	Titrant	Sample	AO Major Contributors	Ref.
TAC	Br_2	Human blood from patients with different pathologies	S-containing amino acids, ascorbic and uric acids, catecholamines, serum albumin, porphyrins	[65,66]
		Human plasma from patients with different pathologies	S-containing amino acids, ascorbic and uric acids, catecholamines, serum albumin	[65–69]
		Tea extracts	Natural phenolic AOs, ascorbic acid	[38]
		Plant raw materials and plant-based medicinal preparations	Natural phenolic AOs, ascorbic acid	[39]
		Juices, balms and *Rhodiola rosea* L. extract	Natural phenolic AOs, ascorbic acid	[70]
		Cognac and brandy	Ellagic and gallic acids, syring- and coniferaldehydes, vanillin, 5-hydroxymethylfurfural	[71]
		Spices micellar extracts	Natural phenolic AOs, capsaicinoids, ascorbic acid	[72]
		Tea and coffee	Natural phenolic AOs, ascorbic acid	[73]
		Marmalade and marshmallow with plant additives	Ascorbic acid, natural phenolic AOs	[74]
		Candy caramel with plant extracts	Ascorbic acid, natural phenolic AOs	[75]
		Extra virgin olive oils	Phenolic compounds	[76]
FRP	$[Fe(CN)_6]^{3-}$	Tea and coffee	Natural phenolic AOs	[77]
		Cognac and brandy	Ellagic and gallic acids	[71]
		Spices micellar extracts	Natural phenolic AOs, ascorbic acid	[72,78]
CRAC	Ce^{4+}	Spices micellar extracts	Natural phenolic AOs, ascorbic acid	[72]

TAC based on the reactions of AOs contained in human blood and its fractions has been successfully used for the monitoring of human antioxidant status [65–69]. The statistically significant changes in the TAC of blood and plasma for patients with chronic renal failure undergoing long-term hemodialysis at different stages of disease were obtained [65]. Statistically significant differences in TAC for venous and arterial blood and TAC for whole blood and plasma were found. The effect of the hemodialysis procedure on the organism antioxidant status was evaluated. The TAC of the blood and plasma from children with all investigated forms of cerebral palsy was statistically significantly decreased vs. control group [66]. The various forms of cerebral palsy were characterized by different TAC values. Among them, the severe forms of disease showed higher TAC than the mild forms. This is explained by violation of the sulfur-containing amino acid metabolism in the case of severe forms of cerebral palsy. This leads to the increase in S-containing amino acids and the disulfide form of glutathione concentration in blood. These compounds react with the titrant and are major contributors to TAC. Plasma TAC has been studied in patients with purulent infections [67], with a different etiology of chronic renal failure [68] and with lung diseases (chronic obstructive pulmonary disease, tuberculosis and lung cancer) [69] and statistically significant difference vs. control group and between the subgroups [67–69] has been observed. Correlations with other parameter-characterizing antioxidant/oxidant status (catalase activity, transition metal contents, low density lipoproteins concentration, oxidation potential) have confirm the accuracy of TAC evaluation.

A wide range of foodstuff and plant materials containing mainly natural phenolic AOs and ascorbic acid has been also studied. Antioxidant parameters can be used for the optimization of extraction of AOs from plant material (spices [71], herbs [39,70]). The statistically significant difference in TAC has been obtained for green and black tea [73], while the FRP for them has been similar due to the presence of albumins in green tea that binds proteins [77]. In the case of cognac and brandy [71], the TAC and FRP are statistically significantly higher for cognacs than for brandies and grow with the age increase for both types of aged distilled beverages. The results of coulometric measurements confirm that antioxidant properties of cognacs and brandies are mainly caused by lignin-derived phenolic AOs extracted from oak barrels. Positive correlations of TAC, FRP and CRAC with standard approaches for the antioxidant properties evaluation (total phenolics by Folin–Ciocalteu, antioxidant activity towards 2,2-diphenyl-1-picrylhydrazyl) confirm the accuracy of the coulometric methods developed [71,72,74].

The nutritional properties of AOs in beverages (juices, balms, tea and coffee) including their changes in the presence of matrix components can be estimated using total antioxidant parameters as shown in the example of tea and coffee polyphenol bioavailability in the presence of milk proteins [73,77].

Another important application point of total antioxidant parameters obtained by coulometric titration is the fast screening of the target samples including their quality control. The only problem to be solved is the necessity of standard or reference samples that should be used in order to build the corresponding reference scale for the samples under investigation. In some cases, this problem is successfully overcome. For instance, coulometrically determined antioxidant capacity using electrogenerated bromine as a titrant allows for the classification of extra virgin olive oils [76]. TAC and FRP parameters can be used for the detection of crude forgery of brandy [79] containing the non-grape nature of spirits and "cocktail" of synthetic and other flavoring agents instead of lignin ethanolysis products (ellagic and gallic acids, syring- and coniferaldehydes, etc.). Moreover, FRP is a more selective parameter because a lesser number of compounds (mainly dihydroxybenzenes) react with the titrant.

The advantages of the coulometric sensor system for the estimation of total antioxidant parameters are the simplicity, rapidity and reliability of the data obtained. Furthermore, coulometric methods, in contrast to spectrophotometry, exclude the usage of unstable reagents like 2,2-diphenyl-1-picrylhydrazyl and can easily be applied for the colored and viscous samples. The high sensitivity of the coulometric titration allows to significantly

decrease the volume of the sample required for the analysis that plays a crucial role in the case of biological fluids (20 μL of blood or plasma is enough for the one measurement [65]).

4. Coulometric Detection of Antioxidants in Chromatography and Flow Systems

As was mentioned above, the combination of high-performance liquid chromatography with coulometric detection (HPLC-CD) is one of the ways to improve the selectivity of AOs quantification in the samples of complex matrix. Coulometric detection is characterized by high sensitivity, low detection limits, lower background currents and less potential interferences, absolute quantification without calibration graph, possibility of trace analysis [80–82]. The application of several electrodes in series (up to sixteen coulometric detectors) allows to build a coulometric array working in a screening mode [83]. In this case, the potential of the first electrode in the sequential configuration of working electrodes is usually 0.2–0.3 V lower than the potential of the second analytical electrode, where the main detection occurs based on oxidation of the separated analytes. This allows the removal of unwanted readily oxidizable components prior reaching the second electrode [84]. The electrodes for coulometric detectors should have large active surface and are most commonly made of a porous carbon. Recently, coulometric detectors with renewable working material of glassy carbon microbeads [85] and carbon felt [86] have been developed. The potentiostatic mode is usually used for the coulometric detection.

HPLC-CD has been successfully applied to isoflavonoids and lignans [87], a wide range of other phenolic [88–98], lipophilic [89,99,100] and S-containing AOs [101–105] (Table 4).

Table 4. Applications of coulometric detection in high-performance liquid chromatographic quantification of AOs.

Antioxidant	Working Electrode	Potential, V	Limit of Detection	Sample	Ref.
Phenolic acids	PGDE	+1.0	0.4–1.1	Model solutions [3]	[89]
Flavonoids	2 PGE	−0.5, +0.8	0.13–1.84 μg mL^{-1}	Orange juice	[90]
Flavones	8 PGE	+0.25–+0.9	No data	Beer	[91]
8 Marker polyphenols	16 PGE	+0.05–+1.0	No data	*Rhizoma Smilacis Glabrae*	[92]
Flavonols and phenolic acids	4 PGE	+0.12, +0.36, +0.48, +0.60	No data	Bilberry, lingonberry, cloudberry and sea-buckthorn berry	[93]
Phenolic AOs	8 PGE	+0.2–+0.9 with 0.1 V step	1.9–25.1 μg L^{-1}	Wines, meads and Japanese knotweed 's roots	[94]
25 Phenolic AOs	8 PGE	+0.2–+0.9 with 0.1 V step	4–29 μg L^{-1}	Meads	[95]
Vanillin Eugenol Thymol Carvacrol	2 PGE	+0.5	0.81 μg L^{-1} 3.0 μg L^{-1} 3.1 μg L^{-1} 1.4 μg L^{-1}	Essential oils for aromatherapy and aromatic herbs for culinary	[96]
Phenolic AOs	4 PGE	+0.25–+0.75	0.03–1.70 ng mL^{-1}	Olive oils	[97]
Phenolic AOs	8 PGE	+0.3, +0.4, +0.5, +0.65, +0.75, +0.8, +0.1, +0.2	1.6–8.3 μg kg^{-1}	Honey	[98]
α-Tocopherol	4 PGE [1]	−0.45, −0.45, −0.45, +0.4	50 pg	Rat plasma or erythrocyte membrane	[99]
Tocopherols	PGDE [2]	+0.5	0.8–2.2 nM	Model solutions [3]	[89]
trans-Lycopene	8 PGE	+0.2–+0.62 with 0.060 V step	50 fmol per 20 μL injection	Human plasma, buccal mucosal cells, prostate and cervical tissue biopsies	[100]
α-Lipoic acid	2 PGE	+0.3–+0.7	0.005 μg mL^{-1}	Dietary supplements	[101]
Reduced glutathione	2 PGE	+0.45, +0.59	1.85 nM	Human plasma	[102]
	4 PGE	+0.25–+0.80	15 fmol	Cultured hepatocytes	[103]
	2 PGE	+0.75, +0.95	2.1 μM	Human blood	[104]

Table 4. *Cont.*

Antioxidant	Working Electrode	Potential, V	Limit of Detection	Sample	Ref.
Captopril	2 PGE	+0.60, +0.95	0.6 μg mL^{-1}	Captopril tablets	[105]
Ascorbic acid	8 PGE	+0.1–+0.4	90 nM	Celaskon tablets, orange, apple and human serum	[106]
	16 PGE	+0.03	164 ng mL^{-1}	Model solution [3]	[107]
	2 PGE	0.0, +0.25	0.025 μM	Mouse and human red blood cells	[108]
Carnosic acid	2 PGE	-0.1, +0.45	1.387 μg L^{-1}	Meat and meat products	[109]

[1] porous graphite electrode; [2] pencil graphite disposable electrode; [3] solution of AO in appropriate solvent.

The limits of detection achieved are impressive (up to the fmol level). Coulometric detection provides lower limits of detection and a wider linear dynamic ranges in comparison to other types of detectors (Table 5) [89,96,101,107]. Multi-analyte detection allows finding the marker AOs in the case of complex samples as well to perform their quality evaluation, recognition and discrimination. The last ones are based on the presence of specific AOs to be considered as typical for the sample under investigation or on the chemometric treatment of experimental data.

Table 5. The comparison of coulometric and other detectors in high-performance liquid chromatographic quantification of AOs.

Antioxidant	Detector	Limit of Detection	Ref.
Eugenol	UV	62 μg mL^{-1}	[96]
	Amperometric	9.7 μg mL^{-1}	
	Coulometric	3.0 μg mL^{-1}	
Vanillin	UV	15 μg mL^{-1}	
	Amperometric	12 μg mL^{-1}	
	Coulometric	0.81 μg mL^{-1}	
Thymol	UV	41 μg mL^{-1}	
	Amperometric	17 μg mL^{-1}	
	Coulometric	1.4 μg mL^{-1}	
Carvacrol	UV	55 μg mL^{-1}	
	Amperometric	13 μg mL^{-1}	
	Coulometric	3.1 μg mL^{-1}	
α-Lipoic acid	UV	0.025 μg mL^{-1}	[101]
	Coulometric	0.005 μg mL^{-1}	
Pyridoxal	Fluorescent	97.2 ng mL^{-1}	[107]
	Coulometric	6.6 ng mL^{-1}	

An original chromatographic approach with coulometric detection has been developed for the monitoring of hydroxyl radical generation formed via Fenton reaction and the estimation of antioxidant and prooxidant capacity of target compounds [110]. The salicylate aromatic hydroxylation derivatives as markers of hydroxyl radicals production have been used. The hydroxyl generation has been performed via Fenton reaction using Fe(II) or Cu(II) ions as catalysts. The AOs acting as both chelators of transition metals and scavengers of hydroxyl radicals have been studied. The tested substances were 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-chloro-7-iodo-8-hydroxyquinoline, catechin, EDTA (only for copper), homovanillic acid, quercetin, phloroglucinol, and trientine (only for copper). Among them, EDTA and trientine act as copper chelators and 5-chloro-7-iodo-8-hydroxyquinoline acts as an iron and copper chelator without iron/copper reducing activity. Quercetin, DOPAC and catechin are chelators with iron/copper reducing activity while phloroglucinol, homovanilic acid and 3-hydroxyphenylacetic acid do not show chelating properties and act as pure AOs. The method developed confirms that the redox behavior of these bioactive molecules can be at least in part related to their capacity to

block or increase hydroxyl radical production via Fenton reaction that is dependent on the AO/transition metal ratio.

There are several examples of combination of flow injection systems with coulometric detection [111–113]. Flow injection with a coulometric array detector is based on the 16 porous graphite-working electrodes poised at potentials from +100 to + 850 mV with increments of 50 mV (vs. Pt reference electrode) and response to the total capsaicinoids contents in chili habanero [111]. The method shows better analytical characteristics than ultra-high performance liquid chromatography with diode array detector as well as a significantly decreased time of analysis from 15 min to 30 s. Further development of this approach has been performed through a coulometric electronic tongue—a device based on one or more low-selective coulometric sensors showing cross-sensitivity towards various compounds in the solution, each contributing to a specific sensory stimulus [112]. The coulometric electronic tongue consisted of 16 porous carbon electrodes positioned in series, each poised at increasing potentials (from + 50 to + 850 mV vs. Pt reference electrode) [112]. The detection potential of + 450 mV was chosen for the total capsaicinoids quantification due to the lowest impact of interferences.

Another electronic tongue based on flow injection coulometry has been developed for the estimation of the antioxidant capacity of fresh lettuce [113]. Similarly to the previous one [112], an electronic tongue was used, consisting of a series of 16 porous carbon electrodes, each poised at a fixed potential from +100 to +850 mV at a step of 50 mV [113]. The system is capable of analyzing up to 60 samples per hour confirming its high throughput. The approach can be used for the characterization and optimization of lettuce extraction conditions as well as the effect of storage (one week at 5 °C) on lettuce.

Thus, electronic tongues based on flow injection coulometry are a quick, comprehensive, and easy-to-handle alternative for the foodstuff and food additives control.

5. Conclusions

Coulometric sensor systems are an attractive and effective tool for the evaluation of antioxidant properties. Both individual AOs, groups of AOs as well as total AO contents in various samples can be quantified under the conditions of coulometric titration with titrants–oxidants or coulometric detection in chromatography or flow injection systems.

Coulometry being an absolute quantitative method significantly simplifies the determination procedure as far as it does not require calibration graphs and standard solutions, respectively. High sensitivity and rapidity, low sample volumes, possibility to use eco-friendly solvents, possibility to measure turbid and colored samples make coulometric sensor systems a perfect alternative to traditional methods for AOs determination, i.e., spectrophotometry and chromatography. Flow injection analysis with coulometric detection provides very fast response (30 s) as well as high throughput (up to 60 samples per hour) in combination with high selectivity of target AO response.

Total antioxidant parameters like TAC, FRP and CRAC obtained by coulometric titration with different titrants–oxidants give the opportunity to control the quality and to characterize the nutritional value of the foodstuff, bioactive compounds and bioadditives that can be used for the further prediction of their effect on the human antioxidant status and health. The TAC of human biofluids (blood, serum and plasma) allows the evaluation of human organism antioxidant status and the effectivity of drug or other types of therapy on the disease treatment.

The further development of coulometric sensor systems based on the application of coulometric titration should be focused on the search for novel titrants that are similar to the reactive oxygen and nitrogen species generated in living systems. The electrogeneration of these species itself would be a great progress in the field. In this case, the conditions of the reaction between AO and titrants should be close to the ones in vivo.

Another trend in the coulometric analysis of AOs is the improvement of the AO response selectivity. This problem can be probably solved using chemically modified electrodes and using titrants that are selective towards target AO. Another way is the

application of selective preliminary extraction or derivatization as well as flow injection systems with coulometric detection. The last ones are of the most interest as far as they show the high throughput of the samples and rapid response in combination with the possibility of excluding the interferences using coulometric electronic tongue or an array mode of detection. Furthermore, the types of AOs can be discriminated by setting certain potentials on the working electrodes.

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