24

Electrochemical Methods in Analysis of Biofuels

André L. Santos[1], Regina M. Takeuchi[1],
Paula G. Fenga[2] and Nelson R. Stradiotto[2]
[1]*Faculdade de Ciências Integradas do Pontal – UFU – Ituiutaba*
[2]*Departamento de Química Analítica – Instituto de Química – UNESP – Araraquara*
Brazil

1. Introduction

Energy is vital for modern society since commercial, agricultural and industrial activities are highly energy-consuming. Moreover, the marvels of the modern technology have enhanced the aspirations of people for an improved quality of life, which is more and more dependent on energy. It is a consensus that this very high energy demand can not be adequately supplied by traditional energy sources such as fossil-based fuels. In addition, there are many concerns about the usage of these fuels in future, mainly taking into account that they are obtained from a finite source and that their production and utilization are processes highly pollutant. These environmental concerns are widely justified taking into consideration, for example, that 56.6 % of anthropogenic green house gases (GHG) emissions of 2004 were associated to fossil fuel use [1]. Thus, for a sustainable future, it is essential to develop integrated energy models linking both traditional and modern renewable energy sources in order to decrease the global dependence on petroleum and to minimize environmental problems associated to its use as energy supply. A very complete discussion focused on the development and evaluation of integrated energy models can be found in the review written by Jebaraj and Iniyan [2].

Among renewable energy sources, biomass has the highest potential to adequately respond to energy requirements from modern society of both developed and developing countries [3]. Biomass is a term for all organic material originated from plants which convert sunlight into plant material through photosynthesis. The term includes all land- and water-based vegetation, as well as all organic wastes. Therefore, biomass resource is the organic matter, in which the energy of sunlight is stored in chemical bonds [4]. Renewable energy is of growing importance in responding to concerns over the environment and the security of energy supplies. In this context, biomass provides the only renewable source of fixed carbon, which is an essential ingredient of our fuels [3]. Biomass can be converted in different forms of useful energy and the literature presents some works especially dedicated to the technologies of biomass conversion [5] including thermochemical conversion processes [6]. The conversion process and its efficiency are influenced by the composition of biomass. Therefore its knowledge is important to select the more appropriated conversion process. A complete discussion about biomass composition and its variability is presented by Vassilev et al [7].

In despite of the advantages of biomass as energy source, some discussions have been done about the potential environmental damages associated with mega-scale biomass production and there are some concerns about its competition with food production [8-11]. These discussions are very important and must be encouraged in scientific community, because they have an important role for the development of responsible, efficient and environmentally correct energetic policies. In the same direction of these discussions, some authors have worked aiming to decrease NO_x emissions of oxygenated biofuels such as biodiesel and ethanol being this subject reviewed by Rajasekar et al [12]. In despite of the concerns associated with global use of biomass to produce energy, there is a consensus in scientific community that if biomass is efficiently produced and processed and if responsible energetic policies are adopted, biomass will represent a very important global source of energy in the future [13]. There are three main political concerns driving bioenergy production today: energy security, global climate change trends and rural incomes [1]. Therefore, bioenergy production involves important sectors of our society such as agricultural, commercial, environmental and industrial. Thus, the efficient and environmentally compatible development of bioenergy requires a stronger emphasis on inter-disciplinary research as stated by Petersen [1].

Bioenergy is especially attractive for tropical or subtropical developing countries because they present ideal climatic conditions to produce biomass. Besides environmental advantages, the use of bioenergy in these countries has also economical advantages because it minimizes their needs of imported petroleum. Some authors present revisions about the use of bioenergy in some developing countries such as Taiwan [14] and Mexico [15] and an interesting discussion about regional added value provided by regional biomass sources is presented by Hoffmann [16]. Bioenergy can be used in three ways: production of liquid transportation fuels, heating and electricity generation. It presents some advantages when compared with other renewable energy sources. For example, bioenergy can be produced continuously in contrast to intermittent wind and solar sources. Moreover, biomass is the only source of high grade renewable heat. However, the possibility of to obtain renewable transportation liquid fuels is the most attractive feature of biomass and a very important application of bioenergy nowadays [17].

Transportation is one of the most energy-consuming activities in modern society. According to International Energy Agency statistics, this sector is responsible for 60% of the world's total oil consumption [18]. Transportation is also almost completely dependent on petroleum-based fuels such as gasoline and diesel [19]. Therefore, transportation sector is very sensible to the several economic limitations associated to petroleum such as its geographically reduced availability and instability of price. In addition, petroleum-based fuels are highly pollutant and nowadays petroleum-based motor vehicles are responsible for more than 70 % of global carbon monoxide emissions and 19 % of global dioxide carbon emissions [18]. These facts clearly show the importance of decreasing the usage or replacing petroleum-based fuels in a near future. Liquid biofuels for transportation have recently attracted attention of different countries due their characteristics such as renewability, sustainability, common availability, regional development, rural manufacturing jobs, reduction of greenhouse gas emissions and biodegradability [20].

The term biofuel is referred to solid, liquid or gaseous fuels that are predominantly produced from biomass. Biofuels include bioethanol, biobutanol, biodiesel, vegetable oils, biomethanol, pyrolysis oils, biogas and biohydrogen. Among biofuels, bioethanol and biodiesel are by far the most used nowadays and many scientists agree that they have potential for replacing petroleum-based fuels [20]. Therefore, it is expected that the demand for biofuels will rise drastically in a near future. Some governmental actions in different countries support this statement. For example, nowadays bioethanol derived from corn responds by approximately 2 % of the total transportation fuel in USA and another 0.01 % is based on biodiesel. The USA Department of Energy has set ambitious goals to replace 30 % of the liquid petroleum transportation fuel with biofuels by 2025 [21]. In this context, there are no doubts that biofuels, mainly bioethanol and biodiesel, will play a very important place in the worldwide energetic policies.

This text is dedicated to the quality control of liquid automotive biofuels by using electroanalytical techniques. Presented discussions will be restricted to bioethanol and biodiesel, the most used transportation liquid biofuels nowadays. More general discussions about biofuels can be found in the literature [22] including the production of other biofuels [23,24], technologies for biofuel production [25,26] economic [27] and environmental [28,29] aspects related to biofuels.

1.1 Bioethanol and biodiesel

Bioethanol is by far the most widely used biofuel for transportation worldwide. Production of bio-ethanol from biomass is one way to reduce both consumption of crude oil and environmental pollution [18]. Besides these advantages, bioethanol has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization, which lead to theoretical efficiency advantages over gasoline in a combustion engine [18].

USA and Brazil are, respectively, the two largest producers of bioethanol. USA have surpassing Brazil since 2005 and in 2008 there were 136 ethanol plants in USA which are able to produced 7.5 billion gallons of ethanol annually [30]. Brazil was pioneering in the usage of bioethanol for transportation in large scale, starting in 1975 when the National Alcohol Fuel Program (ProAlcool) was initiated. ProAlcool was a response from Brazilian government to petroleum crises occurred in 1973. Ethanol fuel was firstly used in Brazil in its anhydrous form as an additive for gasoline and its use has banned lead from Brazilian gasoline. Nowadays, besides the use of anhydrous ethanol as additive, hydrated ethanol is used as fuel in its pure form or blended with gasoline in any proportion in flex-fuel engines. The large scale use of bioethanol has brought several advantages to Brazilian society, including reduced dependence of imported petroleum, jobs generation, acceleration of rural economy, developing of production technologies besides environmental advantages such as substantial reduction of GHG emissions. The interior of São Paulo State is one of the most rich and developed areas in Brazil. In this region, ethanol production is the main agricultural and industrial activity which demonstrates the positive impact of ethanol usage in Brazilian economy.

Most of the bioethanol produced in the world derives from plant juice containing sucrose from sugarcane in Brazil and starch from corn in the United States [31]. Ethanol can also be produced from sugars derived from lignocellulosic material by hydrolysis of the cell wall using enzymes, physical and chemical treatments. Ethanol obtained for this process is called second generation ethanol. The efficiency of the lignocellulose conversion process has not yet proven to be economical, but second generation ethanol is a highly desired goal because it could significantly broaden the choice of feedstock which would contribute to avoid competition between ethanol and food production. Several aspects of bioethanol production are discussed in literature including technologies to produce anhydrous ethanol [32], biotechnological procedures [33], alternative feedstocks [34], pretreatments for efficient enzymatic hydrolysis [35] and works totally devoted to second generation bioethanol [36-39].

Biodiesel is the second most used liquid biofuel worldwide. It is obtained from renewable sources such as vegetable oils and animal fats. Its biodegradability, nontoxic nature and low emissions profile are the main attractive features of this biofuel [40]. The performance of biodiesel as transportation fuel is very similar to petrodiesel [41], which makes biodiesel the only biofuel potentially able to replace petrodiesel. Some characteristics of biodiesel such as its low sulfur content and its lubricating property make it a fuel even better than petrodiesel [41]. However, before the use of biodiesel as a fuel in its pure form some of its properties must be improved such as its viscosity, oxidation resistance and crystallization temperature [42].

Germany and France are the largest producers of biodiesel [43] being Europe responsible for 70 % of the biodiesel produced in the world. European biodiesel production has drastically increased from 1998 to 2008. In this period, biodiesel production grew from 475 thousand tons to 16 million tons [42]. In USA the production of biodiesel has increased from 2 million gallons to 700 million gallons in the period 2000-2008. A significant increase on biodiesel fuel market is expected not only in developed but also in developing countries such as China, Brazil, Indonesia and Malaysia [42]. Nowadays the main usage of biodiesel is as an additive to petrodiesel forming blends containing 5-10 % of biodiesel [42]. The percentage of biodiesel depends on regulatory legislation of each country and there is a global tendency in to increase biodiesel contents in these blends.

Biodiesel is produced by a transesterification reaction between triacylglycerides from renewable biological resources, such as plant oils or animal fats and a short-chain alcohol such as methanol or ethanol. Therefore, biodiesel is a mixture of monoalkyl esters of fatty acids (mostly methyl or ethyl esters) [42]. The main side product in the production of biodiesel is glycerin which after additional purification could be used in perfumery, medicine, and in the microbiological synthesis of ethanol, succinic acid, etc. [42]. There are three main transesterification processes that can be used to produce biodiesel: base-catalyzed, acid-catalyzed and enzyme-catalyzed transesterification [43]. The raw material used as oil source for biodiesel production includes edible and non-edible oils, algae, waste cooking oil, etc. [41]. There are several vegetable oils used as raw material for biodiesel production. In the United States and Brazil soybean oil is a source that is already scaled up for biodiesel production. Nevertheless, other sources, such as rapeseed (in Europe), sunflower, peanut, cotton, palm oil, coconut, babassu, and especially castor oil, may also be used in different parts of the world once their cultivation can achieve an economic up-scaling [43]. The use of edible oil and arable food land to produce biodiesel is very controversial and it has been considered the main drawback of biofuels. Thus there is a huge interest in the use of alternative raw materials for biodiesel production. Several scientists have worked to develop new producing processes in which algae can be used as raw material [44,45]. However, several challenges need to be overcome in order to make possible the commercial production of biodiesel from algae at a scale sufficient to make a significant contribution to our transport energy needs.

1.2 Quality control of biofuels

According to discussed in previous sections, the importance of biofuels for world energetic future is evident. Therefore, the assurance of the properties and quality of biofuels is a key aspect to their successful commercialization and market acceptance. Besides economic aspects, the quality of biofuels has implications over environmental aspects since some contaminants can lead to severe operational problems which can increase the emissions levels of engines using biofuels or reduce the efficiency of the biofuel. Moreover, some contaminants can accelerate undesirable reactions leading to storage instability. The composition of biofuels is highly variable because of several factors such as variability of raw materials, influence of climate and soil, transportation and storage method, etc. This fact reinforces the importance of rigid international technical specifications able to guarantee uniform quality parameters for biofuels produced in different regions. However, technical specifications are not the only requirement for an efficient quality control. The quality control of biofuels depends also on reliable official analytical methods and certified reference materials. Reliable official analytical methods are the tool responsible to guarantee that technical specifications are obeyed while certified reference materials are extremely important to validate and check the performance of both official and new analytical methods [46].

Quality control of biofuels is a complex and interdisciplinary issue that represents a real challenge to our society. In order to respond to this challenge there are necessary integrated actions involving scientific and political sectors. International integrated actions seem to be the best way to assure the homogeneity of the quality of biofuels produced in different regions of the world. Fortunately, these actions are in progress since 2007 when Brazil, European Union (EU) and USA, the three largest biofuels producers, elaborated a White Paper on internationally compatible biofuel standards issued by a tripartite task force. USA, EU and Brazil agree that there are different standards for biofuels which were known to be an obstacle to the free circulation of biofuels among the three regions. It is stated on the White Paper that the participants agree to promote, whenever possible, the compatibility of biofuels-related standards in their respective regions. Such compatibility would not only facilitate the increasing use of biofuels in each of the regional markets, but also would support both exporters and importers of biofuels by helping to avoid adverse trade implications in a global market [47].

The subsequent subsections of this work will be focused in the contribution of analytical chemistry for quality control of bioethanol and biodiesel. Especial attention will be addressed to official analytical methods and technical specifications adopted by EU, USA and Brazil. Some attention will be also addressed to alternative non-electrochemical analytical methods. Despite this work is centered on electroanalytical methods, some references about other analytical techniques will be included in order to provide additional material for readers interested in more general aspects of analytical chemistry of bioethanol and biodiesel.

1.2.1 Bioethanol quality control

The main bioethanol producers have important regulatory agencies which are responsible to implement technical specifications and official norms for quality control of bioethanol. In Brazil, the agency responsible for the inspection and commercialization rules of all fuels is ANP (National Agency of Petroleum, Natural Gas and Biofuels). ANP resolutions establish criteria of quality control using Brazilian Norms (NBR) which are defined by ABNT (Brazilian Association of Technical Norms). In USA and EU the quality of bioethanol is regulated, respectively, by ASTM (American Society for Testing and Materials) and ECS (European Committee for Standardization). ECS resolutions are ruled by European Norms (EN). Technical specifications adopted in EU, USA and Brazil regulate some physical properties of bioethanol, such as appearance and density and also the content of some organic and inorganic species. The complete list of technical specifications adopted by the three main producers of bioethanol is presented in Table 1.

Quality parameter	USA		Brazil		EU
	ASTM D4806-10	ASTM D4806-10 Undenatured	ANP36/2005 Anhydrous	ANP36/2005 Hydrous	EN15376-07
Appearance	Clear & Bright	Clear & Bright	Clear & no impurities	Clear & no impurities	Clear & Bright
Color	Dye allowed, but not mandated	Dye allowed, mandated but not mandated	Dye mandated for in country, but not for export.	Dye prohibited for in country	Dye allowed, but not mandated
Maximum density at 20 ºC, kg/m^3	----	----	791.5	807.6	----
Maximum electrical conductivity, µS/m	----	----	500	500	----
Maximum acidity, mg/L	0.007	0.0074	0.0038	0.0038	0.007
pHe	6.5-9.0	6.5-9.0	----	6.0-8.0	Dropped
Minimal ethanol Content, vol.%	92.1	93.9	99.6	----	[96.8]
Minimal ethanol + C3-C5 saturated alcohols content, vol.%	----	[98.4]	----	----	98.8
Minimal total Alcohol content, vol.%	----	[98.95]	99.6	95.1	[99.76]
Maximum saturated C3-C5 alcohols content, vol.%	----	[4.5]	----	----	2.0
Maximum water content, vol.%	1.0	1.05	[0.4]	[4.9]	0.24
Maximum methanol content, vol.%	0.5	0.53	----	----	1.0
Minimal/Maximum denaturant content, vol%	1.96/5.0	No denaturant	No denaturant	No denaturant	Set by country 0/1.3
Maximum Hydrocarbons content, vol.%	----	----	3	3	----
Maximum solvent-washed gum content, mg/100 mL	5.0	5.3	----	----	----

Maximum gum or residue by evaporation, mg/100mL	5 (washed gum)	5.3 (washed gum)	----	5 (unwashed)	10 (unwashed)
Maximum sulfate content mg/kg	4	4.2	----	4	----
Maximum chloride content mg/kg	40	42.1	----	1	25
Maximum sodium content mg/kg	----	----	----	2	----
Maximum copper content, mg/kg	0.1	0.105	0.07	----	0.1
Maximum iron content, mg/kg	----	----	----	5	----
Maximum phosphorus content, mg/L	----	----	----	----	0.5
Maximum sulfur content, mg/kg	30	5	----	----	10

Numbers in [ ] are calculated estimates and not specified limits

Table 1. Ethanol specifications adopted in USA, Brazil and EU [47]

Table 1 shows that technical specifications are very similar in the three regions and therefore they do not constitute serious commercial barriers. On the White Paper, technical specifications were classified in three groups: Category A – similar specifications; Category B – specifications with significant difference but which can be aligned and Category C – specifications very different which can not be aligned in short term. Among the ethanol technical specifications, only water content was classified in category C, thus if Brazil and USA exporters wish to supply EU market additional drying will be required [47]. The similarity on technical specifications will be improved even more, since these three regions are working together in this direction since 2007. This will significantly contribute to homogenize the quality of bioethanol produced in different regions which is very important to the consolidation of this fuel as an important product in the global market.

Regulated parameters are those able to seriously compromise the general quality of bioethanol causing economical or environmental damages. For example, high values of acidity, conductivity, water content and extreme pH values can intensify the corrosion power of ethanol. Therefore, if these parameters are not properly regulated, ethanol fuel could cause fast deterioration of metallic components present in the production process, transportation, storage and engines using ethanol fuel. Some chemical species such as sodium, chloride and sulfate are also able to intensify the corrosion power of ethanol and, therefore, they must be regulated as well. Copper and iron can catalyze polymerization reactions in blends ethanol-gasoline; these reactions promote the formation of gum and sediments which seriously affects the performance and cause several damages in the engine and fuel injection system. Sulfur constitutes an example of contaminant able to increase the emission level of ethanol fuel, since after combustion it is converted in SO_x which are released for atmosphere, thus the regulation of sulfur content is important from environmental concerns.

Regulated Specie	Official Analytical Method	Technical Norm
Chloride	Ion Chromatography	ASTM D7319-09 NBR 10894-07 EN 15492-08
Sulfate	Ion Chromatography	ASTM D7319-09 NBR 10894-07 EN 15492-08
Sodium	Flame Photometry	NBR 10422-07 EU and USA have no specification
Copper	Atomic Absorption Spectroscopy (AAS)	ASTM D1688-07 NBR 11331-07 EN 15488-07
Iron	Atomic Absorption Spectroscopy	NBR 11331-07 EU and USA have no specification
Sulfur	X-ray Fluorescence Spectrometry (USA)	ASTM D2622-10
	Ultraviolet Fluorescence* (USA)	ASTM D5453-09
	X-ray Fluorescence Spectrometry (EU)	EN 15485-07
	Ultraviolet Fluoresce (EU)	EN 15485-07
		Brazil has no specification
Phosphorous	Spectrophotometry (EU)*	EN 15487-07 Brazil and USA have no specification
Methanol	Gas Chromatography (EU and USA)	ASTM D5501-09 EN 13132-00 Brazil has no specification
C3-C5 alcohols	Gas Chromatography (EU)	EN 13132-00 Brazil and USA have no specification

* After reaction with ammonium molybdate

Table 2. Official analytical methods adopted in Brazil, USA and EU for quantification of some inorganic and organic contaminants of bioethanol [47,49]

To ensure that required technical specifications are fulfilled there are necessary reliable official analytical methods and their development constitutes the main contribution of Analytical Chemistry community to bioethanol quality control. Official analytical methods are established by technical norms which specify all conditions of analyses including analytical technique, sample pretreatment, calibration procedure, etc. The main analytical official methods used to regulate the content of inorganic and organic contaminants in bioethanol fuel are presented in Table 2.

From Table 2 it can be observed that official analytical methods adopted for bioethanol quality control are predominantly instrumental ones, which are usually characterized by high analytical performance showing high sensitivity, selectivity and analytical frequency. Most of technical norms are very recent and they present updated analytical methods. In the last five years it was observed an impressive evolution in some specifications. For example, sulfate specification was initially based on a gravimetric method, afterwards a volumetric method was adopted and more recently the official analytical method adopted by Brazil, EU and USA is based on ion chromatography, a very modern and reliable analytical technique. Chloride analytical official method has also evolved from volumetric for an ion chromatography method which is also adopted by the three regions [46]. The use of modern instrumentation and analytical techniques is important to ensure that precise and exact analytical data will be obtained which is the basis for a reliable quality control. On the other hand, the use of modern and more expensive analytical instrumentation usually faces resistance from producers who believe these investments are unnecessary. Regulatory agencies has fulfilled they role to overcome such resistances by adopting high performance and modern analytical method for the quality control of bioethanol. This is very important in order to guarantee that ethanol will take its place as a key global product in a very near future.

Although bioethanol technical specifications are modern, they still have some problems which have attracted the attention of several researches. Spitzer et al [48] have presented a very interesting discussion about pH and conductivity determination in ethanol fuel including metrological problems associated with these parameters. High ethanol and low water containing solutions typically lead to difficulties in pH determination with unstable readings, long response times and device depending results. These are indications for low conductivity of the sample, dehydration of the glass electrode membrane and large junction potentials. Due to a lack on ethanol based reference buffer solution, the pH electrode in all test methods is calibrated in aqueous standard buffer solutions which is still far from being satisfactory. Therefore, worldwide harmonization in pH and conductivity measurement practices is urgently needed for reliable results as stated by Spitzer et al. [48].

Another limitation of bioethanol technical specifications is that they contemplate only a few numbers of chemical species. For example there is no specification for acetaldehyde which is a well known ethanol fuel contaminant. Higher aldehydes which can decrease ethanol storage stability are not regulated as well. This fact has encouraged many scientists to develop alternative analytical methods for quantification of several chemical species in ethanol fuel. Most of these alternative analytical methods are instrumental ones and they bring interesting refinements in bioethanol analysis. The most complete work about alternative analytical methods for bioethanol fuel analysis was written recently by Oliveira et al [49]. This work revises the main advances in the quantification of organic and inorganic species in ethanol fuel by using modern analytical techniques. From this work it can be concluded that gas chromatography is predominant for the quantification of organic contaminants in bioethanol followed by high performance liquid chromatography (HPLC). Atomic spectroscopy techniques are the most used for the quantification of cationic inorganic contaminants of bioethanol. For analysis of anionic inorganic it is observed a large variety of analytical methods such as electrophoresis, UV-spectroscopy, besides potentiometric and conductometric methods. Since Oliveira's work provides a very comprehensive and recent revision about the use of modern alternative analytical methods for analysis of bioethanol, this topic will not be covered here.

1.2.2 Biodiesel quality control

The same regulatory agencies responsible for bioethanol specifications in Brazil, EU and USA are also responsible for specifications of biodiesel in these three regions. As stated previously, biodiesel is produced from a transesterification reaction between a vegetal oil or fat and an alcohol, mainly methanol or ethanol in presence of a catalyst, usually a strong base such as sodium or potassium hydroxide. The transesterification reaction plays a crucial role for the final quality of biodiesel because side products, residues of catalyst and reagents are the main sources of contamination of the final product. Besides glycerol, the main side product of transesterification reaction, mono-, di- and triacylglycerol can be also formed since this reaction is a stepwise process [50]. Therefore, the content of glycerols in biodiesel must be limited by specification since these species brings several operational problems causing loss of efficiency and damages in the engines. Besides glycerols other contaminants are introduced in the transesterification step such as Na, K, free fatty acids and alcohols and all of them have deleterious effect on final quality of biodiesel. Therefore these species are regulated by technical specifications. The complete list of specifications of biodiesel adopted in Brazil, EU and USA is presented in Table 3.

Biodiesel specifications adopted in Brazil and USA are applicable for both fatty acid methyl esters (FAME) and fatty acid ethyl esters (FAEE), whereas the current European biodiesel standard is only applicable for FAME. Also, the specifications for biodiesel in Brazil and USA are used to describe a product that represents a blending component in conventional petroleum based diesel, while the European biodiesel standard describes a product that can be used either as a stand-alone diesel fuel or as a blending component in conventional petroleum based diesel [47]. Taking into consideration these facts it is not surprising that there are some significant differences among the sets of specifications adopted in Brazil, EU and USA as can be observed in Table 3.

Table 3 clearly shows that biodiesel specifications are not so closely aligned in the three regions as bioethanol specifications. Contrasting with bioethanol, biodiesel is not a single compound but it is a mixture of fatty acids esters which makes biodiesel more complex than bioethanol. In addition, biodiesel can be produced via methylic or ethylic route, originating two chemically different kinds of biodiesel: FAME and FAEE mixtures. These features associated with the fact of biodiesel is produced from several types of feedstock on different regions also lead to significant variations in the performance and characteristics of the final product making a very hard challenge to develop a common set of specifications for biodiesel produced in different regions. On the White Paper, the biodiesel tripartite task force has identified ten specifications classified in category C [47], which demonstrates a significant divergence in specifications and properties of biodiesel produced in the three regions. This could be seen as a strong impediment to trade. However, in most of the cases, it is possible to meet different regional specifications by blending various types of biodiesel to achieve the desired quality and specifications [47] which drastically minimize trade problems.

Quality parameter	Limits			
	Units	USA ASTM D6751/2003	EU EN14214/2003	Brazil ANP 07/2008
Ester Content	% mass	----	96.5 min[a]	96.5 min
Density at 15 ºC	Kg/m^3	----	860-900	----
Density at 20 ºC	Kg/m^3	----	----	850-900
Kinematic viscosity at 40 ºC	mm^2/s	1.9-6.0	3.5-5.0	3.0-6.0
Distillation Temperature, 90 % Recovered	ºC	360 max[b]	----	360 max
Oxidation Stability, 110 ºC	hours	3.0 min	6.0 min	6.0 min
Flash Point	ºC	130.0 min	120 min	100 min
Cetane Number		47 min	51 min	Report
Cloud Point	ºC	Report	----	----
Cold Filter Plugging Point	ºC	----	5 max (Grade A) 0 max (Grade B) -5 max (Grade C) -10 max (Grade D) -15 max (Grade E) -20 max Grade F)	19 max
Total Contamination	mg/Kg	----	24 max	24 max
Carbon Residue (on 100 % sample)	% mass	0.050 max	----	0.050 max
Acid Number	mg KOH/g	0.50 max	0.50 max	0.50 max
Water and Sediment	% volume	0.050 max	----	0.050 max
Water Content	mg/Kg	----	500 max	500 max
Methanol or Ethanol Content	% mass	0.20 max	0.20 max	0.20 max
Free Glycerol	% mass	0.02 max	0.02 max	0.02 max
Monoacylglycerol Content	% mass	----	0.80 max	Report
Diacylglycerol Content	% mass	----	0.20 max	Report
Triacylglycerol Content	% mass	----	0.20 max	Report
Total Glycerol	% mass	0.24 max	0.25 max	0.25 max
Linolenic Acid Methyl Ester	% mass	----	12.0 max	----
Polyunsaturated Methyl Ester	% mass	----	1 max	----
Iodine Value	g I_2/100 g	----	120 max	Report
Sulfated Ash	% mass	0.020 max	0.02 max	0.02 max
Na + K	mg/kg	5 max	5 max	5 max
Ca + Mg	mg/kg	5 max	5 max	5 max
Copper Strip Corrosion	Rating	Class 3	Class 1	Class 1
Phosphorous Content	% mass	0.001 max	0.0010 max	0.0010 max
Sulfur Content	mg/Kg	15/500 max	10 max	50 max

[a]Minimal value necessary, [b]Maximum value allowed

Table 3. Biodiesel specifications adopted in USA, Brazil and EU [47]

Another consequence of the higher complexity and variability of the chemical composition of biodiesel is the need of a large number of specifications for chemical contaminants. This is necessary because biodiesel presents more chemical species together which increases the possibility of chemical processes able to compromise the quality of this biofuel. For example, free glycerol may separate from biodiesel and to accumulate on the bottom of storage containers or vehicle fuel tank attracting other polar components such as water, monoglycerides and soaps. These agglomerates can result in damage to the vehicle fuel injection system due to impairment of fuel filters. Alkali and alkali-earth are also able to damage engines and fuel injection systems because they can promote the formation of ash and soaps. Alkali metals are introduced in biodiesel composition from alkali catalyst residue while alkali-earth metals can be introduced from hard washing water [47]. The content of ethanol or methanol is another very important specification because these alcohols can significantly accelerate corrosion of metallic components of engines; they can cause adverse effects on injectors due to its high volatility and low lubricity [47]. The corrosion power of biodiesel can be also increased by the presence of excess of free acids, which is expressed by the acid number. This parameter is also useful to indicate the degree of fuel ageing during storage because the acid number increases due biodiesel degradation promoted mainly by esters hydrolysis reactions, originating free fatty acids. Thus, acid number is a very important quality parameter which is limited by technical specifications in Brazil, EU and USA. The ability of biodiesel to cause corrosion of copper, zinc and bronze parts of the engine and the storage tank is evaluated by the copper strip corrosion. To determine this quality parameter, a copper strip is heated to 50°C in a biodiesel bath for three hours, and then compared to standard strips to determine the degree of corrosion. This corrosion resulting from biodiesel might be induced by some sulfur compounds and by acids, so this parameter is correlated with acid number [47]. Water is other important chemical contaminant of biodiesel because high water contents promote biological growing, causing slime formation compromising fuel filters and lines in production systems. Moreover, high water contents promote biodiesel hydrolysis reaction originating free fatty acids, increasing the acid number and the corrosion power of biodiesel. Therefore, water content is directly related to storage stability of biodiesel since water is able to degrade biodiesel by hydrolysis reaction. A review of the test methods used to evaluate the stability of biodiesel was recently written by Jain and Sharma [51]. Other chemical contaminants whose contents are limited by specifications are phosphorus and sulfur. Phosphorus can reduce the efficiency of exhaust catalytic system of vehicles and sulfur increases the SO_x emissions [47]. Therefore, both contaminants cause environmental damages and their contents must be regulated.

Some of the specifications of biodiesel (Table 3) are indicative of its purity such as ester content, density, distillation temperature and total contamination [47]. Other specifications are directly related to biodiesel performance as fuel. For example, the cetane number describes biodiesel propensity to combust under certain conditions of pressure and temperature. High cetane number is associated with rapid engine starting and smooth combustion. Low cetane number causes deterioration in this behavior and causes higher emissions of hydrocarbons and particulate. In general, biodiesel has slightly higher cetane number than fossil diesel. Cetane number increases by increasing length of both fatty acid chain and ester groups, while it is inversely related to the number of double bonds [47]. The number of double bonds can be evaluated by the iodine number, linolenic acid methyl ester and polyunsaturated methyl ester content and also by oxidation stability. Kinematic viscosity is another important quality parameter for fuels because high viscosity compromises the spray injected in the engine and at low temperatures high viscosity can damage fuel pumps. Flash point is a measure of flammability of a fuel and therefore it is an important safety criterion in transport and storage. Flash point values for biodiesel are usually double those observed for petroleum-based diesel which represents a safety advantage of biodiesel [47]. Thus, the set of biodiesel specifications adopted in Brazil, EU and USA are able to guarantee both the purity and the suitable performance of biodiesel. More detailed discussion about ASTM and EN technical specifications can be found in the literature [50,52].

Analytical official methods used for the determination of the content of the main inorganic and organic contaminants of biodiesel are presented in Table 4.

From Table 4 it can be observed that official analytical methods adopted for biodiesel quality control are also predominantly instrumental ones. The complexity of biodiesel requires highly selective and sensitive analytical methods, because there are several chemical species together in the sample and the contaminants are minor components of biodiesel, therefore they must be reliably quantified even at low concentrations. These requirements are filled only by instrumental analytical methods. Analogously to the observed for bioethanol, technical specifications of biodiesel are very modern and they are based on updated instrumental analytical methods. From Table 4 it is possible to observe that gas chromatography is the only analytical technique used for quantification of organic contaminants of biodiesel. For the quantification of inorganic contaminants, there is predominance of atomic spectroscopic methods, which are characterized by extremely high selectivity and sensitivity. Despite the extremely high analytical performance of ICPOES, the high instrumental costs and the needing of highly specialized operators are strong limitations for its use in routine analysis. In order to minimize problems associated with very expensive analysis, there are still active technical norms for inorganic contaminants based on AAS, a high sensitive, selective and instrumentally simpler technique than ICPOES. Therefore, for quantification of Na, K, Ca and Mg technical regulation adopted in Brazil, EU and USA allows the use of both techniques.

There are two very important works about analysis of biodiesel: the review written by Knoth in 2006 [50] and the review written by Monteiro in 2008 [52]. A more recent review about quality and analytical methods for biodiesel analysis was written by Lôbo et al [53] in 2009. All these works bring comprehensive discussions about both official and alternative analytical methods employed for biodiesel analysis. From these works it is possible to conclude that organic contaminants of biodiesel are quantified mainly by gas chromatography (GC), which is the base not only of official but also of alternative analytical methods. Alternative GC-based methods have been also used for monitoring the transesterification reaction [52]. Flame ionization detector (FID) is the most used detection system for GC-biodiesel analysis; however the use of coupled mass spectrometer (MS) has increased lately. This detection system significantly increases the identification power of GC, avoiding ambiguities about the chemical nature of the eluting species [52]. Recent approaches for development of GC-based alternative analytical methods include the use of ethyl oleate as internal standard for glycerol determination [54], the use of ionic liquid as stationary phase for determination of FAME in diesel blends [55] and the determination of polyunsaturated FAMEs with wax capillary column using methyl tricosanoate as internal standard. [56]. Two-dimensional gas chromatography has also been successfully used for analysis of diesel blends [57] and for determination of fatty acid mixtures composition [58]. These methods have brought interesting advances on determination of organic contaminants of biodiesel.

Regulated Specie	Analytical Official Method	Technical Norm
Water + Sediment	Centrifugation	ASTM D2709-96
Water	Karl Fisher (Coulometry)	EN 12937-01 ASTM D6304-07
Methanol or Ethanol	Gas Chromatography	EN 14110-03 NBR 15343-08
Free Glycerol	Gas Chromatography	ASTM D6584-10 EN 14105-03 EN 14106-03 NBR 15341-06 NBR 15771-09
Total Glycerol	Gas Chromatography	ASTM D6584-10 EN 14105-03 NBR 15344-10
Linolenic Acid Methyl Ester	Gas Chromatography	EN 14103-03
H^+ (acid number)	Potentiometric Titration	ASTM D664-09 NBR14448-09
	Visual indicator Titration	EN 14104-03
Na + K	Atomic Absorption Spectroscopy	EN 14108-03 EN 14109-03 NBR 15554-08 NBR 15555-08 UOP* 391
	Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES)	EN 14538-06 NBR 15553-08
Ca + Mg	Inductively Coupled Plasma Optical Emission Spectroscopy	EN 14538-06 NBR 15553-08
	Atomic Absorption Spectroscopy	ASTM D4951-09 EN 14107-03 NBR 15556-08

Phosphorus Content	Inductively Coupled Plasma Optical Emission Spectroscopy	ASTM D4951-09 EN 14107-03 NBR 15553-08
Sulfur Content	Ultraviolet Fluorescence Spectrometry	ASTM D5453-09 EN 20884-10 EN 20846-10

* Universal Oil Products

Table 4. Official analytical methods adopted in Brazil, USA and EU for quantification of some inorganic and organic contaminants of biodiesel [47]

Liquid chromatography (LC) methods are less common than GC for quantification of organic contaminants of biodiesel. However, according to some authors, HPLC methods are able to provide short time analysis with no need of chemical derivatisation. HPLC is very useful to study the transesterification reaction because it is compatible with gradient elution, which is necessary to the efficient separation between mono-, di- and triacylglycerols [50,52]. Santori et al [59] have proposed a liquid chromatographic method which successfully enables the monitoring of transesterification reaction. Other recent examples of biodiesel analysis by LC methods include determination of acylglicerols, free fatty acids and FAME by size exclusion chromatography [60] and determination of ester in glycerol phase after transesterification by a RP-LC method [61].

Besides chromatographic methods, some spectroscopic techniques have been also used to study the transesterification reaction and to determine organic contaminants in biodiesel. Among these methods, nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectrometry are the most commonly employed [52]. NMR was successfully used by Nagy et al [62] to evaluate both the yield of transesterification and the overall quality of biodiesel by a procedure based on glycerol hydroxyl phosphitylation followed by ^{31}P-NMR analysis. Other applications of NMR to biodiesel analysis include determination of biodiesel levels in blends [63]; determination of free fatty acids [64] and FAME [65] in biodiesel samples and also evaluation of the biodiesel preparation process [66]. An analytical method using FTIR spectroscopy has been developed by Mahamuni and Adewuyi [67] to determine biodiesel content in the reaction mixture, which allows to monitor the transesterification reaction. Moreover, this method could be successfully employed to determine the biodiesel content in petrodiesel-biodiesel blends. These authors have also shown that small modification in the analytical method enables its application to detect biodiesel adulteration with soy oil. Chuck et al [68] have performed an interesting study by using three analytical techniques: UV, FTIR and refractive index to analyze the chemical identity of biodiesel and its content in blends. These authors have concluded that FTIR is suitable to determine biodiesel content in biodiesel-petrodiesel blends while refractive index is very useful to determine the biodiesel identity. These authors also concluded that UV Spectrophotometry can be successfully used for determination of total amount of polyunsaturated esters in samples of B100 biodiesel.

An interesting advance in biodiesel analysis was introduced by Abdelnur et al [69]. These authors have described a very simple and efficient alternative method for biodiesel typification by using mass spectrometry. This method allowed the analysis of biodiesel without necessity of any sample pretreatment which is a great advantage over most of official and alternative analytical methods. Another example of using mass spectrometry for biodiesel analysis can be found in the work by Prates et al [70]. These authors have combined mass spectrometry with multivariate calibration to analyze biodiesel-petrodiesel blends. The proposed method was a simple, fast and reliable way for quantification of biodiesel in fuel blends.

Some authors have used Flow Injection Analysis (FIA) systems to analyze biodiesel. Pinzi et al [71] have proposed a FIA method with spectrophotometric detection based on determination of free and bound glycerol to monitor biodiesel production. This method was based on liquid–liquid extraction of glycerol from the biodiesel to an aqueous ethanolic phase in which glycerol was oxidized to formaldehyde with meta periodate with subsequent reaction with acetylacetone. The reaction product was photometrically measured at 410 nm. The analytical performance of the proposed method was compared with EN 14105 technical norm at a 95 % confidence level and no statistical difference between the results obtained by both methods was observed. The main advantages of the proposed method are: high analytical frequency, low cost (compared with GC and HPLC) and minimizing of sample handling. Rio et al [72] have used a Sequential Injection Analysis (SIA) based flow titration to determine the total acidity of biodiesel samples. A diode array spectrophotometric detector was used linked to chemometric tools. The results obtained with the proposed method were in good agreement with those obtained by official analytical methods, demonstrating the reliability of the method. Moreover, the use of a SIA method helps to reduce the amounts of sample, reagents and time consumed.

Analysis of inorganic contaminants of biodiesel has been performed mainly by atomic spectrophotometric methods and several recent works about it are available in the literature. Lyra et al [73] have used flame atomic absorption spectrometry (FAAS) to develop an analytical method for quantification of Na, K, Ca and Mg in biodiesel samples. In order to overcome problems associated with the high viscosity of biodiesel, the authors have prepared a microemulsion as sample pretreatment procedure. This procedure has allowed the reliable quantification of the metallic species in biodiesel, which was demonstrated by the concordance between the results obtained by the proposed method and those obtained by the official NBR 15556 technical norm. The preparation of a microemulsion was also successfully used by Jesus et al [74] for determination of Ca and Mg in biodiesel samples by FAAS. The use of microemulsions is a very interesting way to make biodiesel compatible with sample introduction systems used in FAAS because microemulsions are prepared in a very fast, simple and inexpensive way. This sample pretreatment strategy was adopted also by Chaves et al [75] for determination of K and Na in biofuel samples by FAAS. Another interesting sample pretreatment procedure is the dry decomposition in muffle furnaces. This procedure was successfully employed by Oliveira et al [76] for determination of Na in biodiesel by flame atomic emission spectrometry. Graphite furnace atomic absorption spectrometry (GF-AAS) has been also successfully used for biodiesel analysis. A GF-AAS method for direct determination of phosphorus in biodiesel samples was proposed by Lyra et al [77]. These authors have used an automatic solid sampling accessory which allowed P determination in biodiesel without any pretreatment or sample dilution. GFAAS has been also used for quantification of heavy metals such as As [78], Cd, Pb and Tl [79] in biodiesel samples. Although the content of these metals is not limited by specification, their high toxicity justifies the interest on their quantification in biodiesel samples. Aranda et al [80] have proposed an analytical method for determination of Hg in biodiesel samples. The samples were introduced directly as oil-in-water emulsions in a flow injection manifold followed by cold vapor generation coupled to atomic fluorescence spectrometry (FI-CV-AFS). The authors were able to quantify both inorganic and organic mercury (as methyl mercury) after irradiation of the sample with a UV source.

2. Electroanalytical methods

Electrochemical techniques are versatile and powerful analytical tools, which are able to provide high sensitivity, low detection limits, associated to the use of inexpensive instrumentation which presents as an additional advantage relatively low operator training requirements. These features make electrochemical techniques very attractive not only for routine analytical applications but also for fundamental physicochemical research such as molecular interactions, or interface processes [81]. Electroanalytical methods involve the measurement of an electrical property such as current, potential, conductivity or electric charge which are directly related to the analyte concentration. Therefore, electroanalysis is based on the fundamental relationships existing between electricity and chemistry and it has been used in several areas such as biomedical analysis, environmental monitoring, industrial quality control, etc. [82]. The most common routine applications of electroanalysis include potentiometric determination of pH, amperometric oxygen and glucose probes, conductometric control of ultra purified water, coulometric quantification of water in non-aqueous materials, etc. [81]. Besides these well established routine applications, the intensive research on electroanalysis has brought new and impressive potential applications for electroanalytical methods. A very interesting example of a new using of electroanalytical methods is found in the work written by Doménech-Carbó [83]. This author discusses the application of electroanalytical methods to quantify analytes from works of art demonstrating that electroanalysis is potentially useful to provide information about conservation and restoration of cultural goods. Another impressive and modern application of electroanalytical methods is the development of amperometric electronic tongues for food quality analysis [84].

Researches on electroanalysis are not limited to develop new applications for electroanalytical methods but they are also interested on the improvement of the analytical performance of electrodes and electrochemical sensors. Thus, special attention has been devoted to the use of new materials for electrode construction. These researches have produced electrochemical devices highly sensitive and selective which have allowed the use of electroanalytical methods even for analysis of very complex samples such as biofuels. These electrodes with superior analytical performance are based on modern materials and their development was achieved mainly due the incorporation of nanotechnology on the electrode production processes. Therefore, several nanomaterials have contributed to developing modern electrodes such as carbon nanotubes [85,86] and several kinds of nanoparticles [87]. The use of zeolites, silicas and nanostructured porous electrodes has also significantly contributed to development of electrodes with improved analytical performance [88,89].

The main electroanalytical techniques are potentiometry, conductometry, coulometry, voltammetry and amperometry. Recent advances and fundamental aspects of these techniques will be briefly discussed here. More complete and deeper fundamental discussions can be found in classical electroanalytical literature [82,90,91].

Potentiometry is based on thermodynamic relationships between the electrical potential measured at equilibrium conditions and the activity of some specie in solution. In electrochemical sense, equilibrium conditions mean that there is no current flowing across to the electrochemical cell. Therefore, in potentiometry, information about composition of a sample is obtained by measuring electrical potential between two electrodes in absence of appreciable current. Analytical applications of potentiometry include the detection of endpoint in potentiometric titrations and direct potentiometry in which the potential measured is used to provide the analyte concentration. The main applications and advances in potentiometric methods involve direct potentiometry, thus only this modality will be discussed here. The whole electrochemical system required for potentiometric measurements includes a potentiometer which allows potential measurements and prevents the flowing of current between the electrodes; a reference electrode whose potential is constant and insensitive to the sample composition and an indicating electrode which potentiometrically responds to the analyte activity [92]. Potentiometry allows accessing activities (or effective concentration) data instead analytical concentration. This is extremely advantageous for fundamental physicochemical studies however, for analytical purposes this is unfavorable since in this case information required is about analytical concentration and not activities. To overcome this, calibration procedures in direct potentiometry must ensure that the activity coefficient of analyte is the same in both sample and calibration standards. Standard addition method is also very useful for direct potentiometric analysis. Thus, if calibration is properly performed, the electrical potential measured between reference and indicating electrodes (with $I = 0$) can be directly related to analyte concentration (in logarithmic form). Membrane based electrodes are the most common indicating electrode used in direct potentiometry. They are also called Ion Selective Electrodes (ISEs) because their high selectivity. The potential developed across the ISE/sample/reference electrode system is not related to redox reactions but it is a junction potential which it is related to specific chemical equilibrium that takes place in the solution/membrane interface such as ion exchange. Useful membranes should present non porous nature, insolubility in the sample solution, little conductivity, high mechanical stability and selective reactivity towards the analyte. The most known membranes are the glass membrane for pH measurements and the crystalline LaF_3 membrane for determination of fluoride. ISEs are very attractive analytical devices because they present fast responses and wide linear range; they are not affected by color or turbidity of sample; they are inexpensive and finally they are not sample destructive [82]. A recent and very complete review about electrochemical theory related to potentiometry was written by Bobacka et al [93]. Modern ISE research is focused mainly in developing new ion recognition species, also called ionophores. Thus several new ionophores have been developed which have produced more stable and selective ISEs. Another trend in ISE research is to develop more robust and practical devices, thus there is significant interest on the development of all-solid state ISEs and miniaturized systems [93]. A very interesting discussion about advances in potentiometric sensors was written by Privett et al [94]. These authors present an impressive number of references discussing the main advances in potentiometry covering ISEs development and analytical applications in several areas. ISEs can be successfully used as detection system in flow analysis combining the high selectivity of these devices with all advantageous features of FIA systems such as low reagent and sample consumption, better repeatability, minimized sample manipulation and consequent lower risk of sample contamination, high analytical frequency, and low instrumentation cost. Trojanowicz [81] has presented a very instructive discussion about the use of potentiometry as detection system in flow analysis and capillary electrophoresis.

The electrical conductivity of a solution can be used for analytical purposes and this is the base of conductometric methods. The solution conductivity depends on the ion concentration and the characteristic mobility of the ions in solution. Therefore, the conductivity of one-solute solution can be used to directly provide information about the concentration of ionic species in solution. However, in multiple-solute solutions the contribution of single ionic specie to the total solution conductivity can not be done and in these situations conductometry is not useful for analytical purposes. This lack of selectivity has discouraged the development of conductometry as a widespread electroanalytical technique. However, conductometry is one of the most sensitive techniques available and therefore its modern applications exploit this high sensitivity in situations in which selectivity is not required. One example of application of conductometry is the monitoring of the quality of ultra purified water. The conductivity in this case is very low and the presence of even extremely low concentrations of ionic impurities causes a drastic increase in conductivity indicating the deterioration of the water. Another modern application of conductometry is as detection system in ion chromatography. In this case, selectivity is not required since ionic species are previously separated by the chromatographic column. Therefore ion chromatography enables the quantification of ionic species at extremely low concentration due the high sensitivity of conductometry. All these applications are based on direct conductometry measurements; however conductometry can be also used to detect endpoint in conductometric titrations. General considerations about conductometric titrations can be found in the literature [92,95]. Conductometry uses a simple, robust and inexpensive instrumentation, which includes an alternating voltage source and a conductance cell composed by two Pt electrodes sealed in only one piece. Alternating voltage is necessary to avoid electrolysis. Conductometric systems are very versatile and they can be constructed in several sizes and shapes which makes conductometric detectors totally adaptable to flow analyses systems.

Coulometric methods are based on the electrolytic reduction or oxidation of the analyte for a time sufficient to ensure its quantitative conversion to a new state of oxidation. Electrolysis can be performed at controlled potential or controlled current conditions. Controlled current electrolysis leads to two kinds of coulometric techniques: electrogravimetry and coulometric titrations. Only this last modality will be discussed here because it represents the main used coulometric technique, general discussions about other coulometric techniques can be found in the literature [91,92,95]. All coulometric methods do not require any calibration procedure because the measured property (electrical charge or mass) is related to the quantity of analyte by fundamental relationships [9091-92,95]. This is advantageous because makes analysis faster and more reliable since errors associated to the preparation of chemical standards are eliminated. Moreover, coulometric methods are the most precise and exact analytical methods available in analytical chemistry [95]. In coulometric titrations the titrant is generated in situ by electrolysis at controlled current. The relationship between electrical charge and the amount of titrant generated is based on Faraday's law of electrolysis [90]. To be used in a coulometric titration, the electrode reaction must satisfy the following requirements: it must be of known stoichiometry and it must occur with 100 % current efficiency [91]. In the same way of classical titrations, coulometric titrations also require methods to detect the endpoint, which can be visual or instrumental [95]. However, coulometric titrations offer a number of advantages over classical titrations: (a) very small amounts of substances can be determined without the use of micro volumes. (b) Standard solutions and standardizations procedures are unnecessary. (c) Substances that are unstable or inconvenient to use because of volatility or reactivity can be employed as titrant since they are generated and consumed instantaneously. (d) Coulometric titrations are easily automated. (e) Dilution effects are absents, making endpoint location simpler [91,95]. Instrumentation required for coulometric titrations is also simple and inexpensive. It consists of inert electrodes, a constant-current source which can be simply a high voltage power supply and a resistor, a coulometer or a timer [91]. Modern applications of coulometric titrations include the determination of uranium [90] and several metallic ions, halides, oxygen and unsaturated organic compounds. However the most common use of coulometric titration is the quantification of water content in non-aqueous liquids, procedure known as Karl-Fischer titration [91,95]. In this method I_2 is electrolytically generated in an appropriated reaction medium in which I_2 suffer a redox reaction involving also water as reagent. Thus, there is a stoichiometric relationship between electrogenerated I_2 and water. The endpoint is detected by using other pair of detector electrodes in an independent circuit. The detector circuit maintains a constant current between the two detector electrodes. At the equivalence point, excess I_2 is produced and it is electrochemically reduced to I^- causing a drastic voltage drop in detector circuit which marks the endpoint. The content of water is an important quality parameter for several products such as edible and lubricating oils, pharmaceutical raw material, food products, industrial solvents and also automotive fuels. Thus, Karl-Fischer analysis is currently widely employed and there are several commercial automated equipments to reliably perform these analyses at a relatively low cost.

Voltammetry is by far the most used electrochemical technique in academic and scientific fields and it is clearly the most useful technique for low-level quantifications. These techniques are based on continuous variation of the potential applied across the electrode-solution interface and the resulting current, which is the property related to the analyte concentration, is recorded. The applied potential, therefore, acts as the drive force for the electrochemical reaction, i.e. reduction or oxidation of the analyte. Cyclic voltammetry (CV) is the most widely used voltammetric technique for acquiring qualitative information about electrode processes. The success of cyclic voltammetry derives from its ability to provide thermodynamic and kinetics data about heterogeneous electron-transfer reactions [82]. CV is usually the first experiment performed in an electroanalytical study, because it offers a fast location of redox potentials of the analyte. Moreover, CV enables the convenient evaluation of the effect of the media, i.e. pH, supporting electrolyte, etc. upon the redox processes of analyte [82].

For analytical purposes, linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) are the most widely used techniques. DPV and SWV are usually preferred due their better discrimination between faradaic and capacitive currents which allows achieving detection limits as low as 10^{-8} mol L^{-1} [90,95]. These different modalities of voltammetric techniques are classified according to the adopted potential-time programs. The basic principles of voltammetric techniques and their respective potential-time programs are extensively discussed on electrochemical books [90-92], articles [96] and in some books specially devoted to voltammetry [97]. Contrasting to coulometric techniques, voltammetry involves extremely low scale electrolysis; therefore, the fraction of analyte oxidized or reduced is negligible. Thus the sample composition remains unchanged after the voltammetric analysis. Another unique feature of voltammetric techniques is that they involve electrolysis in conditions of total limitation by mass transport, thus diffusion of analyte from the body of the solution to the electrode/solution interface plays a crucial role in voltammetric analysis [90].

Comparatively to previously mentioned electrochemical techniques, voltammetry requires the most sophisticated instrumentation, which consists in a potentiostat and a three-electrode electrochemical cell. A potentiostat is necessary to maintain a controlled potential between reference and working electrode without allowing current passage. The potentiostat also presents a secondary circuit connecting working to auxiliary electrode and this circuit is responsible for all current flowing through the electrochemical cell. Auxiliary electrode, therefore, is used just to make possible current flowing. Auxiliary electrodes are constituted by inert and highly conductive materials, such as Pt, stainless steel, etc. Reference electrode is the key to achieve controlled potential conditions and therefore it is a very important component in voltammetric analysis. The literature presents some works totally devoted to reference electrodes including discussions about practical problems associated to them [98] and new techniques for the fabrication of more robust all solid state and miniaturized reference electrodes [99].

Working electrode is the electrode in which the analyte suffers electrochemical reduction or oxidation providing the current that is proportional to its concentration. Several materials can be used as working electrode in voltammetric techniques and they can be divided in two main groups: mercury electrodes, for example: dropping mercury electrode (DME) and hang dropping mercury electrode (HDME) and solid electrodes such as: platinum, gold, glassy carbon, carbon paste electrodes (CPEs), etc. DME has historical importance in electrochemistry, since it was directly involved in the development of polarography by Heyrovsky [49] being this technique the precursor of the modern voltammetric techniques. In 2009 it was celebrated the 50th anniversary of Heyrovsky's Nobel Prize for polarography and a celebrative review was written by Barek [100] this author was also involved in a very important and complete historical review about polarography written in 2002, year in which was celebrated eighty years of the discovery of polarography [101]. Despite its historical importance, the use of DME has decreased lately due mainly to environmental reasons and practical limitation for on-line and *in situ* analyses. Gradually, DME has been replaced by solid working electrodes. Among solid electrodes, glassy carbon (GC) is the most employed in electroanalysis because of its excellent mechanical and electrical properties, wide useful potential range and chemically inert nature. However, other kinds of carbon, such as: graphite, reticulated glassy carbon, pyrolytic carbon and more recently carbon nanotubes [86] also occupy an important place in electroanalysis. Therefore, carbon-based working electrodes are the most widely used in modern voltammetry and a review about the contributions of carbon materials for the development of electrochemical sensors was written by Qureshi et al [102]. Another very important review about advanced carbon electrode materials was written by McCreery [103].

Other very important kind of carbon-based electrodes widely used in voltammetry are the CPEs. These electrodes are constituted by a mixture between carbon powder and a water immiscible organic liquid, which acts as binder agent (pasting liquid). CPEs have become one of the most popular electrode materials used for the laboratory preparation of various electrodes, sensors, and detectors. Therefore, CPEs are used in practically all areas of fundamental and applied electrochemistry including modern electroanalysis. The success of CPE can be attributed to its very attractive physicochemical and electrochemical properties.

The advantageous features of CPEs include low residual current, wide useful potential window, chemical inertness, ease surface renewal, low cost and easiness of prepare. However, the most attractive feature of CPE is the easiness to perform a chemical modification of the electrode. A CPE can be modified simply adding the chemical modifier to the mixture carbon powder/binder agent with no need of specific interactions between the chemical modifier and the electrode material. The chemical modification of a CPE modulates its chemical and/or physical properties establishing specific interactions between the analyte and the electrode surface leading to an electrode with improved analytical performance, i.e. more sensitive and/or selective. In the year 2008, it was exactly fifty years since Professor Ralph Norman Adams has introduced the CPEs. Svancara and a team of very important experts in electroanalysis/electrochemistry have written a very instructive celebrative review [104]. These authors are also responsible for the most complete and recent work about CPEs in which the main advances, trends on CPEs development and application are extensively discussed [105].

Chemically modified electrodes (CMEs) are particularly attractive kind of electrode. CMEs are prepared by immobilization of chemical species onto electrode surface aiming to modulate its properties in order to establish specific interactions between analyte and electrodic surface. Electrode surfaces are usually chemically modified by one of these methods: adsorption (chemical or physical), covalent bond, polymer film coating or via composite preparation. CMEs correspond to a powerful and versatile approach to develop electrodes with analytical performance superior to conventional ones. These electrodes have been bringing impressive advances in electroanalysis and reviews about the preparation and application of these devices are available in the literature [106].

The sensitivity of voltammetric techniques can be significantly improved by using a preconcentration step previously to the voltammetric scan, this procedure characterizes the called stripping voltammetric techniques. The preconcentration step makes use of specific interactions between analyte and electrode surface, which can involves non-electrochemical processes such as adsorption or electrochemical ones such as electrodeposition. Stripping voltammetric techniques are characterized by very low detection limits, extremely high sensitivity, multielement and speciation capability, suitability for on site and in situ applications and minimal sample treatment requirements. The high sensitivity and consequently the low detection limits obtained with stripping analysis techniques are consequences of the preconcentration step, which enhances the analyte concentration at electrode surface. More general and fundamental discussions about stripping voltammetric techniques can be found in the electrochemical literature [82,90,91].

An important advance in voltammetric techniques is the use of microelectrodes which usually present dimensions not greater than 25 µm. Compared with conventional dimension electrodes, microelectrodes present several attractive features, such as improvement of mass transport due to radial diffusion, enhancement of signal–noise ratio, low background currents and immunity to ohmic drop. This last characteristic is very attractive, since it enables the use of microelectrodes in high resistive media without the necessity of supporting electrolyte, minimizing the possibility of chemical contamination of samples. A limitation of using microelectrodes is the extremely low currents recorded with require very sensitive equipments and very powerful amplification systems. In order to overcome this limitation, microelectrodes arrays has been extensively studied and used for development of electrochemical sensors. In these arrays, currents recorded at each individual microelectrode are additive, therefore, microelectrode arrays combine the attractive properties of microelectrodes with macro scale current values leading to very high sensitivity. Xie et al [107] have presented a very interesting review about the use of microelectrodes for quantification of metallic species in water samples and a recent review about the use of microelectrode arrays to develop amperometric sensor was written by Ordeig et al [108]. Classical literature of electrochemistry also brings information about microelectrodes and microelectrode arrays [82,90,91].

Another very interesting electrochemical technique based on potential-current relationships is amperometry. In this technique, the working electrode potential is kept at a controlled and invariable value which is just sufficient to promote the electrochemical reduction or oxidation of analyte. In these conditions, the current flowing through the electrochemical cell will be directly proportional to the analyte concentration. The instrumentation and the electrodes used in amperometric techniques are exactly the same used for voltammetry. Therefore, amperometry also involves electrolysis of minimal quantity of the analyte under condition of total limitation by mass transport. The combination of amperometry with hydrodynamic conditions is a very interesting alternative to develop electroanalytical methods. Hydrodynamic conditions strongly improve the mass transport due to the forced convection and as a consequence, higher current values are obtained allowing the development of more sensitive electroanalytical methods. Another attractive feature presented by amperometry in hydrodynamic condition is the shorter time of analysis.

A great characteristic of all electroanalytical techniques is their total compatibility with flowing conditions which makes them extremely attractive to be used in detection systems in FIA, liquid chromatography and capillary electrophoresis. The combination of electrochemical detection with liquid chromatography (LC-ED) is a powerful analytical strategy which has proven to be one of the more efficient options when attempting to characterize complex matrices. LC-ED is, therefore, widely used for the trace determination of easily oxidizable and reducible organic and inorganic compounds in complex matrices, providing detection limits as low as 0.1 pmol for a number of electroactive compounds. The electrochemical cell and electrodes in LC detector system can be constructed in many different designs according to the convenience. All electroanalytical technique can be employed in LC detection, however a large prevalence of amperometry is observed mainly due its operational simplicity. Additional fundamental information about LC-ED can be found in classical electroanalytical literature [90] and a review about the use of carbon electrodes as detectors in LC is also available [109]. Capillary electrophoresis is a very powerful technique used for separation of charged species. The required apparatus consists of a fused silica capillary filled with a buffer solution. A very high potential (usually 10 - 40 kV) is applied across the capillary and the sample is introduced in an extremity of the capillary while the detection system is located in another extremity. Due to the ionized silanol groups on the capillary walls an electroosmotic flow is produced which causes the elution of all compounds at the cathode, regardless their charge. Compounds are separated based on their differences in electrophoretic mobility; thus, usually the order of elution is faster cations, slower cations, neutral species and finally anions. Electrochemical detection is very advantageous in capillary electrophoresis because it is based on a reaction at electrode surface, therefore the electrochemical cell volume can be extremely small without loss of sensitivity [90]. Modern aspects of electrochemical detection coupled to capillary electrophoresis are discussed in the work written by Trojanowicz [81].

3. Electroanalytical techniques applied to bioethanol and biodiesel quality control

Electroanalytical techniques are adopted in both official and alternative methods used for bioethanol and biodiesel quality control. Official analytical methods exploit mainly those electroanalytical techniques whose instrumentation is simple and inexpensive in which there is no necessity of highly specialized operators. This is expected since official analytical methods are used as routine analyses which must be simple, practical and easily performed. On the other hand, alternative electroanalytical-based methods usually adopt advanced electroanalytical techniques and sophisticated instrumentation. Thus, these methods bring the main advances in electroanalytical field. They are characterized by superior analytical performance, presenting extremely low detection limits associated with very high sensitivity and selectivity. In the next sections, official and alternative electroanalytical-based methods used for bioethanol and biodiesel analysis will be revised and discussed.

3.1 Official electroanalytical-based methods

Important quality parameters of both bioethanol and biodiesel are regulated by technical norms based on potentiometry, conductometry and coulometry. These electroanalytical techniques are preferred due their simplicity, low cost, reliability associated with a low consume of time and reagents. Currently, there are no official analytical methods based on voltammetry or amperometry.

The pH of bioethanol is a very important quality parameter associated to its corrosion power. This quality parameter is regulated by technical norms based on direct potentiometry by using a glass electrode. In Brazil and USA technical norms describing the potentiometric determination of pH of bioethanol are, respectively, NBR 10891-06 and ASTM D6423-08. According to previously discussed, there are metrological problems associated to the determination of pH of bioethanol, since the glass electrode calibration is performed with aqueous buffers while bioethanol is predominantly a non-aqueous system [48]. It is interesting to observe that NBR 10891-06 explicitly describes that there is no correlation between pH values obtained for bioethanol and pH of aqueous solutions. However, this same norm sets that the electrode calibration must be performed by using aqueous standard buffer solutions which is very questionable. Some researches have worked in order to overcome these metrological problems by performing extensive studies about the behavior of glass electrodes in ethanol media [110]. These studies have allowed the introduction of correction factors which is extremely useful to improve the reliability of pH values determined in bioethanol contributing to the development of new and more appropriated technical norms. Direct potentiometry is also the base of a European technical norm (EN 15484-07) that specifies the content of chloride in ethanol fuel by using a chloride-ISE. Potentiometric titration is also used as official analytical method adopted for determination of acid number of biodiesel in Brazil, EU and USA. Technical norms that regulate this quality parameter are NBR 14448-09, EN 14104-03 and ASTM D664-09. In potentiometric titration, the calibration of glass electrode is not critical, because this technique uses relative and not absolute potential values, therefore, all those problems associated with calibration in direct potentiometry are absent in potentiometric titration. This makes technical norms based on potentiometric titration more reliable than those based on direct potentiometry.

As previously discussed, the main modern application of coulometry is for quantification of water content in non-aqueous media by Karl-Fischer titration. This electroanalytical technique is the base of the technical norms adopted in EU (EN 15489-07) and USA (ASTM E1064-08) for determination of water content in bioethanol. Currently, Brazil does not regulate water content in this biofuel. Karl-Fischer titration is also used as official analytical method to determine the water content in the biodiesel produced in Brazil, EU and USA. The respective technical norms adopted in EU and USA are, respectively, EN 12937-01 and ASTM D6304-07. Brazil, currently adopt the European norm to regulate water content in its produced biodiesel.

Conductometry is used as official analytical method for specification of three quality parameters of bioethanol and one quality parameter of biodiesel. Therefore, conductometry is the most used electroanalytical technique for bioethanol and biodiesel quality control. Conductivity itself is a specified property of bioethanol regulated in Brazil by technical norm NBR 10547-06. Currently EU and USA have no specification for bioethanol conductivity but they are considering adding a specification for this property [47]. This is a very important specification because conductivity can easily and quickly determine if bioethanol is contaminated by ionic species, including those that are not specified. In addition, conductivity values are also related to corrosion power of bioethanol, i.e. high conductivity values are associated with high corrosion power. Therefore, conductivity is a very useful property able to provide information about the overall quality of bioethanol. Moreover conductivity measures require a very simple and inexpensive instrumentation and the test is very ease to perform. The other two specified quality parameters of bioethanol regulated by official analytical methods based on conductometry are chloride and sulfate content. Brazil, EU and USA have specifications for the content of these anions; the adopted technical norms are, respectively, NBR 10894-07, EN 15492 and ASTM D7319-09. All these technical norms use ion chromatography with conductometric detection as official analytical method. This is an example of the success of coupling chromatographic separation with electrochemical detection.

A very interesting use of conductometry is in the evaluation of the oxidation stability of biodiesel. This property is specified in Brazil, EU and USA and these three regions adopted the European technical norm EN 14112-03. The oxidation stability of biodiesel is a crucial parameter to ensure correct fuel performance in the vehicles, as well as in storage and distribution. EN 14112-03 adopts the rancimat procedure to determine oxidative stability of biodiesel. Rancimat is a totally automated accelerated oxidation test carried out at elevated temperature and under exposure of air. In rancimat procedure, a stream of purified air is passed through the heated sample (usually 3-6 g of biodiesel) and it is subsequently bubbled through a vessel containing deionized water in which there is a conductivity cell continuously monitoring the system. The resulting oxidation products, i.e. volatile organic acids are carried by the air stream from the sample to the deionized water. When the produced organic acids reach deionized water, their dissociation equilibria are established producing carboxylate anions and H+. The presence of these ionic species causes an increase in conductivity of the water. Generally, oxidation is slow at first stages and it quickly accelerates after its initiation. The point in which the maximum change of rate of oxidation is obtained is called induction time and it is detected by a large and abrupt increase on the conductivity. EN 14112-03 defines a minimal induction time of 6 h at 110 ºC. The oxidative stability obtained at 110 ºC can be extrapolated to provide stability data under storage and transportation temperature. Rancimat procedure constitutes a very smart use of conductometry and this method is also used to evaluate oxidative stability of raw material and products from pharmaceutical and food industry. Rancimat procedure has been also used to evaluate the performance of antioxidant agents. Therefore, conductometry is a very important electroanalytical technique widely used for quality control of several materials.

3.2 Alternative electroanalytical-based methods

The number of alternative electroanalytical-based methods used for bioethanol and biodiesel analysis is higher than the official electronalytical-based methods as shown in Table 5.

From Table 5 it can be observed that the number of chemical species analyzed by alternative electroanalytical-based methods is higher than those whose contents are regulated by official technical norms. This demonstrates that electroanalytical community is not only concerned about impurities that constitute a problem for biofuels quality today but it is also concerned about species which may become a future problem for the quality and commercialization of bioethanol and biodiesel. Table 5 also shows the prevalence of voltammetry and stripping voltammetric techniques as base for alternative electroanalytical methods which is expected since modern voltammetric techniques are very suitable for low-level quantifications. Therefore, most of alternative electroanalytical methods are characterized by very low detection limits.

Direct potentiometry is especially attractive to determine chemical species which can not be easily oxidized or reduced such as alkali metals. Thus, some recent works have described the use of direct potentiometry to determine potassium in biodiesel samples. Two strategies have been adopted in these works: the use of commercial K^+-ISE which is protected from direct contact with biodiesel sample and the development of new indicating electrodes. The first strategy was used by Rapta et al [112] which have employed a PVC membrane-based commercial K^+-ISE to quantify potassium in FAME samples. In order to prevent electrode swelling promoted by FAME, two compartments were used one of them was filled with distilled water and the other was filled with FAME sample. Both compartments were separated by a semi-permeable membrane, constituted by a 20 μm thickness cellulose acetate film. Indicating and reference electrode were immersed in the compartment containing distilled water. The cellulose acetate membrane is permeable to K^+ allowing its extraction from FAME compartment to the aqueous compartment in which K^+ was potentiometrically quantified. This procedure was very efficient to avoid electrode deterioration from exposure to FAME sample which was demonstrated by the good reproducibility and concordance between the results obtained by the proposed method and those obtained by official AAS analytical method. Castilho and Stradiotto [111] have used the second strategy to determine K^+ in biodiesel samples. These authors have developed a new K^+-ISE based on a nickel-hexacyanoferrate film which was deposited onto glassy carbon by cyclic voltammetry in presence of both ions $Fe(CN)_6^{3-}$ and Ni^{2+}. These electrodes were successfully used for quantification of K^+ in biodiesel samples after a liquid-liquid extraction using HCl 0.1 mol L^{-1} as aqueous phase. The developed electrode has presented a near Nernstian behavior and a very satisfactory selectivity with selectivity coefficient for Na^+, NH_4^+ and Li^+ equal to 0.01; 0.5 and 0.0089, respectively. In both works, direct potentiometry has allowed K^+ quantification in biodiesel samples in a very reliable, fast, simple and inexpensive way. These features make direct potentiometry very useful, for example, to evaluate the efficiency of biodiesel final washing procedure for the elimination of KOH used as catalyst in transesterification reaction.

Biofuel	Technique	Analyte	Detection Limit	Reference
Biodiesel	Direct potentiometry	K^+	19 µmol L^{-1}	[111]
Biodiesel	Direct potentiometry	K^+	0.4 ppm	[112]
Ethanol	Direct potentiometry	Cu^{2+}	----	[113]
Ethanol	Conductometric titration	Total Acidity Cl^-	3.0 mg L^{-1} 0.88 mg L^{-1}	[114]
Ethanol	Conductometry/Ion chromatography	Cl^- and SO_4^{2-}	----	[115]
Biodiesel	Conductometry/Ion chromatography	Alkaline Earth and Alkaline metal-ions	----	[116]
Ethanol	Conductometry/Capillary electrophoresis	Inorganic cations and anions	----	[117]
Ethanol	SWV	SO-7 dye	0.09 µmol L^{-1}	[118]
Ethanol	SWV	SB-14 dye	0.29 µmol L^{-1}	[119]
Ethanol	SWV	SB-14 dye	93 nmol L^{-1}	[120]
Ethanol	DPV	Acetaldehyde	0.81 µmol L^{-1}	[121]
Ethanol	SWV	Acetaldehyde	0.24 µmol L^{-1}	[122]
Ethanol	DPV	Acetaldehyde	2.0 µmol L^{-1}	[123]
Ethanol	CV	Furfuraldehyde	0.70 mmol L^{-1}	[124]
Biodiesel	LSV	Glycerol	25 µmol L^{-1}	[125]
Biodiesel	LSV	tert-butylhydroquinone	71 nmol L^{-1}	[126]
Ethanol	Stripping voltammetry-DPV	Cu^{2+}	31 nmol L^{-1}	[127]
Ethanol	Stripping voltammetry-LSV	Cu^{2+}	22 nmol L^{-1}	[128]
Ethanol	Stripping voltammetry-DPV	Cu^{2+}	39 nmol L^{-1}	[129]
Ethanol	Stripping voltammetry-SWV	Cu^{2+} Pb^{2+}	120 ng L^{-1} 235 ng L^{-1}	[130]
Ethanol	Stripping voltammetry-DPV	Zn^{2+} Cu^{2+} Pb^{2+}	0.24 µmol L^{-1} 0.88 µmol L^{-1} 0.66 µmol L^{-1}	[131]
Ethanol	Stripping voltammetry -DPV	Pb^{2+}	7.2 nmol L^{-1}	[132]
Ethanol	Stripping voltammetry-DPV	Ni^{2+}	2.0 nmol L^{-1}	[133]
Ethanol	Stripping voltammetry-DPV	Ni^{2+}	2.7 nmol L^{-1}	[134]
Ethanol	Stripping voltammetry-SWV	Fe^{3+}	2.4 µmol L^{-1}	[135]
Ethanol	Stripping voltammetry-LSV	Zn^{2+}	0.26 µmol L^{-1}	[136]
Ethanol	Stripping voltammetry-LSV	Cl^-	0.13 mg L^{-1}	[137]
Ethanol	Stripping voltammetry-DPV	Sulphur	15 ng g^{-1}	[138]
Biodiesel	Potentiometric stripping	Cu^{2+}	200 ng g^{-1}	[139]
Biodiesel	Amperometry-enzymatic	Free and total glycerol	1x10^{-5} (w/v)	[140]
Ethanol	Amperometric detection-HPLC	Aldehydes Ketones	1.7 ng mL^{-1} 2.0 ng mL^{-1}	[141]
Ethanol	Amperometric detection-HPLC	Acetaldehyde	3.80 µg L^{-1}	[142]
Ethanol	Amperometric detection-HPLC	SB-14 dye	6.4 nmol L^{-1}	[143]
Ethanol	Amperometric detection-FIA	Cl^-	3.7 µmol L^{-1}	[144]

Table 5. Alternative electroanalytical-based methods used for bioethanol and biodiesel analysis

Conductometry coupled to ion chromatography has been successfully used for simultaneous quantification of several inorganic ions in both bioethanol and biodiesel [115,116] which demonstrates the versatility and analytical power of combining chromatographic separation and conductometric detection. Conductometric detection in ion chromatography and capillary electrophoresis represents a very advanced use of conductivity measures. The main approach currently adopted in these detection systems is contactless conductivity detection (CCD) in which electrodes are electrically isolated from the sample being the measurements performed at high frequency conditions. One of the advantageous features of CCD is the total absence of electrode fouling effect since electrode is not in direct contact with sample solutions. Deeper discussions about CCD are beyond of the scope of this work. Additional information about the principles and application of CCD can be found in the original works introducing CCD as detection system in capillary electrophoresis [145,146]. CCD coupled to capillary electrophoresis was used by Muñoz et al [117] for the successful and simultaneous quantification of several cations (Na^+, K^+, NH_4^+, Ca^{2+} and Mg^{2+} using Li^+ as internal standard) and anions (SO_4^{2-}, Cl^- and NO_3^- using lactate as internal standard) directly in commercial ethanol fuel samples. The authors have used a homemade capillary electrophoresis equipment which has presented many advantageous features such as low cost, shorter time analysis and high analytical performance with low detection limits and high sensitivity. Classical conductometric methods have been also employed for ethanol fuel analysis. Avelar et al [114] have used conductometric titration for determining chloride content and free acidity in hydrated ethanol fuel samples. The authors have observed that conductometric titration allows the reliable quantification of total acidity and chloride content in hydrated ethanol fuel being the obtained results in good agreement with those obtained by official analytical methods. Therefore conductometric titration could be successfully used for a simple, inexpensive and fast evaluation of free acidity and chloride content which are corrosive agents representing very important quality parameters for ethanol fuel.

From Table 5 it is possible to observe a large number of voltammetric and stripping voltammetric-based analytical methods which are used mainly for ethanol fuel analysis. Voltammetric based analytical methods are used mainly for determination of organic compounds while stripping voltammetric-based methods are used mainly for quantification of transition metal ions. Working electrodes used in voltammetric methods are mainly based on carbon materials, such as glassy carbon [118,119,121,124], screen-printed carbon electrode [120] and carbon paste electrode [126]. In stripping voltammetric-based methods there is a prevalence of chemically modified carbon paste electrodes which are characterized by a superior ability to preconcentrate the analyte at electrode surface.

Organic species determined in ethanol fuel by voltammetric methods are mainly dye markers and aldehydes. Dye markers are usually purposely added to fuels in order to guarantee their identity and discourage adulterations, thus these dyes are important to quality control of fuels. Two very important dyes marker are solvent blue 14 (SB-14) and solvent orange 7 (SO-7) and the literature has shown that both can be successfully quantified by voltammetry in ethanol fuel samples. Trindade and Zanoni [119] have developed an analytical method for quantification of SB-14 in ethanol fuel samples based on SWV using a glassy carbon working electrode. The authors have performed all voltammetric measurements in a mixture Britton-Robinson (B.R) buffer: dimethylformamide (1:1 v:v).

Under the optimized experimental conditions, SB-14 has presented a very intense and defined cathodic voltammetric peak at -0.4 V vs. Ag/AgCl/$KCl_{saturated}$ which has allowed the sensitive and reliable quantification of this dye in commercial ethanol fuel samples. In order to improve even more the analytical performance of this analytical method, these same authors have replaced the glassy carbon working electrode by a screen-printed carbon electrode [120], making the voltammetric method more practical and inexpensive. The main advantages of screen-printed electrodes are their modest cost, portability and above of all their simplicity of use since only one small arrangement contains working, auxiliary and reference electrodes. Another improvement introduced in the SWV-quantification of SB-14 was the use of an anionic surfactant which has increased the solubility of SB-14 minimizing the quantity of dimethylformamide required in electrochemical cell from 50% to 30% (v:v). This is extremely advantageous since dimethylformamide is a relatively toxic and expensive reagent. The use of the surfactant has brought other benefits to the analytical method, since its presence has tripled peak current values. Moreover, according to the authors, the surfactant forms a charged film on the electrode surface acting as an anti-fouling agent. All these positive factors have allowed lowering detection limit from 2.9×10^{-7} mol L^{-1} (using glassy carbon in absence of surfactant) to 9.3×10^{-8} mol L^{-1} (using screen-printed electrode in presence of surfactant). The most impressive advantageous feature of these methods is their ability to quantify SB-14 directly in ethanol fuel samples without any sample pretreatment which is extremely attractive because it avoids time-consuming pretreatment procedures making the analytical method faster, simpler, less expensive and more reliable. This same group has yet contributed to the field of electroanalysis of dye markers by developing a voltammetric method for quantification of SO-7 directly in ethanol fuel samples [118]. This voltammetric method has also presented excellent analytical performance associated with high simplicity and low cost.

Ethanol fuel is produced by a distillation process; thus its main organic contaminants are those ones more volatile than it such as acetaldehyde. Whereas that acetaldehyde is a toxic air contaminant able to cause lung irritation and that it is a possibly carcinogenic agent, the quantification of acetaldehyde has attracted much attention. Several analytical methods have been proposed for quantification of acetaldehyde in ethanol fuel including voltammetric ones. However, voltammetric determination of aldehydes is difficult due the extremely high potential values required for promote their electrochemical reduction or oxidation. The strategy used to make possible to quantify acetaldehyde by using voltammetric techniques is to adopt a chemical derivatisation procedure before voltammetric analysis. The chemical derivatisation step is responsible to form a more easily oxidizable or reducible product which can be detected voltammetrically. Thus, acetaldehyde has been quantified by voltammetry as imine derivatives [123] and after derivatisation with hydrazine [122] and 2,4-Dinitrophenylhydrazine [121]. In all these cases there is the formation of the group RR´C=N- which is easily reduced to the correspondent amine [147]. All these voltammetric methods allow the quantification of acetaldehyde in a very sensitive way with low detection limits. The only working proposing a direct voltammetric method for quantification of an aldehyde without chemical derivatisation was presented by Saczk et al [124]. These authors have studied the voltammetric reduction of furfuraldehyde in ethanol media using a glassy carbon electrode. The authors have observed that furfuraldehyde presents a well defined cathodic peak which has allowed its reliable quantification in ethanol.

Only two works involving voltammetric analysis of biodiesel were found in the literature. The first one is the work by Lourenço and Stradiotto [125] which have used a Pt electrode to quantify free glycerol in biodiesel samples. The analytical method is based on electrocatalytic oxidation of glycerol promoted by PtO_x electrogenerated onto electrode surface. Before the voltammetric analysis the samples were submitted to a simple pretreatment consisting of a liquid-liquid extraction with water followed by elution of aqueous phase on a C-18 cartridge for elimination of interfering species. Voltammetric scans were recorded in $HClO_4$ solutions and very well defined voltammetric peaks associated to glycerol electrooxidation were obtained. The proposed method has presented a linear range from 56 to 560 mg Kg^{-1}, which is appropriated for biodiesel analysis since its maximum free glycerol content allowed is 200 mg Kg^{-1}. The reliability of the proposed method was demonstrated by addition-recovery experiments performed at a glycerol-free biodiesel sample and the recovery percentages were always close to 100 %. The second voltammetric method used for biodiesel analysis was proposed by Araújo et al [126] for determination of the antioxidant tertbutylhydroquinone (TBHQ). The poor oxidative stability of biodiesel is considered the main drawback to its use in large scale as alternative fuel. Thus several studies have been conducted in order to increase oxidative stability of biodiesel by adding antioxidant agents and TBHQ have yielded good results. Thus, in future the addition of TBHQ in biodiesel may become mandatory, and its content would be controlled by technical specifications. In this context, the development of analytical methods for quantification of TBHQ in biodiesel samples is quite important. Araújo et al [126] have developed a voltammetric method to quantify TBHQ by using a carbon paste electrode and performing voltammetric measurements in B.R. buffer solutions containing the surfactant cetyltrimethylammonium bromide (CTAB). They have observed that the presence of CTAB significantly intensifies the voltammetric peak associated to TBHQ oxidation increasing the sensitivity of the voltammetric method. Addition-recovery experiments were performed in spiked biodiesel samples and recovery percentages were always close to 100 % showing that the proposed method is very reliable. Moreover, the method has allowed TBHQ quantification directly in soybean biodiesel samples by simply diluting them in methanol without any additional pretreatment procedure. Therefore, this method can be seen as a potential analytical approach for future routine quantification of TBHQ in biodiesel samples.

Stripping voltammetric-based methods are the most sensitive electroanalytical methods employed for bioethanol and biodiesel analysis. These methods have been used mainly for quantification of metal ions in ethanol fuel samples and they have presented extremely low detection limits which usually are in the range of nmol L^{-1}. Most of stripping voltammetric methods employed for bioethanol analysis use chemically modified CPEs, which have significantly contributed for electroanalysis of biofuels. Among chemical modifiers used for constructing CPEs, organofunctionalized silica has received especial attention due mainly to the extremely advantageous features presented by silica such as high adsorption capacity, chemical and mechanical stability, insolubility in virtually all solvents and possibility of functionalization with a large variety of functional groups. CPEs chemically modified with organofunctionalized silica have been successfully used for quantification of Cu^{2+} [127,129] and Ni^{2+} [133] in ethanol fuel samples by using anodic stripping voltammetry. In these works CPEs were chemically modified with silica organofunctionalized with aminothiol groups which are able to efficiently preconcentrate metallic ions by complexation with their

S and N atoms. These works brought another important advance for development of CPEs for ethanol fuel analysis since they have produced more rigid and stable composites by replacing mineral oil, the binder agent used in conventional CPEs, by alternative binders. Conventional CPEs are totally inadequate to be used directly in ethanol fuel samples because they are instable in non-aqueous media due dissolution of binder agent which leads to CPE disintegration. Solid paraffin and castor oil-derived polyurethane have been successfully used as agent binder for the quantification of metal ions in ethanol fuel samples [127,129,133]. Both binder agents are very attractive because they are able to produce carbon composites with better mechanical resistance. Moreover these binder agents present low cost and they are easily manipulated allowing preparing the electrodes in a fast, simple and inexpensive way. Solid paraffin-based CPEs were totally stable in ethanol media allowing the quantification of Cu^{2+} [127] and Ni^{2+} [133] directly in the ethanol fuel samples without any pretreatment or dilution step. Polyurethane-based CPE was successfully used by Cesarino et al [129] for quantification of Cu^{2+} in ethanol fuel samples after mixing them with 30% (v:v) of a KNO_3 aqueous solution. Detection limits for all these works are in the range of nmol L^{-1} and their results were in good agreement with those obtained by official analytical methods demonstrating that they are able to provide Cu^{2+} and Ni^{2+} quantification in ethanol fuel samples in a very sensitive and reliable way. Other chemical modifiers used to prepare CPEs for determination of metal ions in ethanol fuel samples include: the anionic-exchange resin Amberlite® IR120 for quantification of Pb^{2+} [132], dimethylglyoxime for quantification of Ni^{2+} [134] and 1,10-ortophenantroline for quantification of Fe^{3+} [135]. All these examples use conventional CPEs; therefore their use was restricted to mixtures ethanol fuel: aqueous electrolyte solution which however has no seriously compromised their overall analytical performance.

Metal-based and metallic electrodes have been also used for quantification of metal ions in ethanol fuel samples by stripping voltammetric methods. Oliveira et al [131] have used a mercury film electrodeposited onto a glassy carbon electrode for simultaneous determination of Zn, Cd, Pb and Cu in ethanol fuel samples. The procedure adopted to prepare the electrodes was very simple consisting in to submit a rotating disc glassy carbon electrode to -0.9 V vs. SCE for 20 min at 200 rpm in a solution containing 2×10^{-5} mol L^{-1} of Hg^{2+}. This electrode was successfully used to determine Zn, Cd, Pb and Cu in ethanol fuel after diluting the samples in aqueous LiCl solution in the ratio 20:80% (v:v), respectively. The authors have used anodic stripping voltammetry and they performed a comparative study between the analytical performance of LSV, DPV and SWV modes. The results have shown that DPV was the most appropriated voltammetric modality which was used to simultaneously quantify Zn, Cd, Pb and Cu in three commercial ethanol fuel samples by using 10 min of electrodepositing time. The obtained results were in good agreement with those obtained by FAAS method, indicating that the proposed method is very reliable. Muñoz and Angnes [130] have used a commercial gold electrode to develop an anodic stripping-SWV method for simultaneous determination of Pb^{2+} and Cu^{2+} in ethanol fuel. The voltammetric measures were performed in a mixture ethanol:water (75:25% v:v) by using 900 s of electrodepositing time. Recovery studies were performed in spiked commercial ethanol fuel samples and recovery percentages were always close to 100 %. A gold microelectrode was used by Takeuchi et al [128] for determination of Cu^{2+} in commercial ethanol fuel samples. These authors have developed a linear sweep anodic stripping voltammetric method using 300 s of electrodeposition time. Not only the preconcentration step but also the voltammetric scan was performed directly in the ethanol sample without any pretreatment. The authors have found that copper in commercial ethanol fuel samples seems to be distributed in its labile and organic complexed forms which can not be efficiently electrodeposited in electrode surface. As a result, the stripping voltammetric method has provided copper contents lower than those obtained by FAAS. The authors have observed that acidification of samples was a very efficient way to recover copper from its complexed forms. After sample acidification, the developed analytical method was successfully employed for Cu^{2+} quantification in six commercial ethanol fuel samples and the obtained results were concordant with those obtained by official FAAS method. This was the first work in literature in which both preconcentration and detection steps were performed directly in the commercial sample by simply adding 1.0 mmol L^{-1} of H_2SO_4. Therefore, this method has allowed the quantification of Cu^{2+} in ethanol fuel samples in a very fast, simple and sensitive way with detection limit of 22 nmol L^{-1}.

The literature also brings stripping voltammetric methods for quantification of non metals in ethanol fuel samples. Ferreira et al [137] have used a HMDE for develop a cathodic stripping LSV method for quantification of chloride in ethanol fuel samples. The accumulation step was performed at +0.15 V vs. $Ag/AgCl/KCl_{saturated}$ potential in which mercury is oxidized forming Hg_2Cl_2. After the accumulation step the potential was swept in negative direction in order to promote the electrochemical reduction of Hg_2Cl_2 which produces a cathodic voltammetric peak providing the analytical signal proportional to chloride concentration. The developed method was employed for chloride quantification in five commercial ethanol fuel samples without any pretreatment or supporting electrolyte addition. The obtained results were concordant with those obtained by a potentiometric comparative method. The proposed method has allowed chloride determination in a very fast way since only 10 s of accumulation time was required. Aleixo et al. [138] have developed a cathodic stripping voltammetric method for determination of total sulfur in ethanol. The analytical method was based on the reduction of sulfur compounds to sulphite using Raney nickel. After that, sulphite was converted to H_2S which was detected by DPV-cathodic stripping voltammetry at a HMDE. The calibration graph covered the concentration range of 1-40 ppb, and the method was successfully employed for determination of sulfur in ethanol.

Almeida et al [139] have presented the only work in which a stripping-based method is used to analyze biodiesel samples. These authors have used constant current potentiometric stripping at a gold electrode to determine Cu^{2+} in biodiesel. In potentiometric stripping analysis (PSA) of a metal ion, the preconcentration step is the same as for voltammetric stripping, that is, the metal is electrodeposited onto electrode surface at constant potential conditions. After the preconcentration step an anodic current (or a chemical oxidant) promotes the metal stripping from electrode surface. As the metal is removed from electrode surface, the working electrode potential varies according to the Nernst equation. The plot of potential as a function of time gives a curve analogous to a normal redox titration curve due the logarithmic term on Nernst equation. A sudden change in the potential (“endpoint”) occurs when all electrodeposited metal has been removed from electrode surface and the time necessary to reach this point is proportional to the bulk concentration of the metal ion [90]. A more convenient peak-shaped response for analytical purposes is obtained by plotting dt/dE vs. E which produces analytical signs in form of peaks which are more convenient. The constant current PSA method used by Almeida et al [139] has allowed the determination of Cu^{2+} in biodiesel without sample decomposition. The analyses were performed after a simple dilution of biodiesel samples in a mixture ethanol-HCl aqueous solution, producing a homogeneous mixture in which Cu^{2+} was directly quantified. The authors have used an electrodeposition potential of 0 V for 300 s and a stripping current of 0.5 µA. These experimental conditions were used to perform Cu^{2+} determination in three spiked commercial biodiesel samples. The obtained results have shown recovery percentages close to 100 % for all analyzed samples, thus PSA has allowed Cu^{2+} quantification in biodiesel samples in a very simple, fast inexpensive and reliable way. Therefore, this work represents a great contribution to electroanalysis of biodiesel, since it is the first one using a stripping method for biodiesel analysis with no need of sample decomposition or extraction procedures.

Amperometry-based analytical methods have been also used for develop alternative analytical methods mainly for determination of organic compounds in bioethanol. The only work describing an amperometric method for biodiesel analysis was recently written by Luetkmeyer et al [140]. These authors have developed an electroenzymatic methodology using an oxygen Clark-type electrode for the determination of free and total glycerol in biodiesel samples. The enzymatic conversion of glycerol consumes oxygen, which is measured amperometrically in a Clark-type electrode and is correlated with the concentration of glycerol in the sample. The analysis of biodiesel samples were performed after a simple liquid-liquid extraction procedure in which glycerol was extracted from biodiesel samples by a mixture containing distilled water, ethanol and heptane. The authors have found a very good correlation between the results obtained by the developed enzymatic amperometric method and the official gas chromatographic method. The proposed method was shown to be promising for the analysis of glycerol in biodiesel samples, with a simpler and inexpensive methodology compared with the gas chromatography technique.

Other examples of using amperometry for bioethanol analysis involve amperometric detection in flow analysis and amperometric detection in liquid chromatography. Paula et al [144] have developed an amperometric-FIA method for quantification of chloride in ethanol fuel samples. These authors have used a glassy carbon electrode chemically modified with a poly-aniline film immobilized onto electrode surface by electrochemical polymerization carried out by cyclic voltammetry. This electrode is able to detect chloride because redox processes of poly-aniline create positive charges inside the polymeric film, these charges must be balanced by negative counter ions from solution. As anions with large hydrated radius can not penetrate in the polymeric film some selectivity is achieve towards small anions such as Cl^-. In the proposed method, the authors have kept work potential at +0.5 V vs. $Ag/AgCl/KCl_{saturated}$ in glycine without adding supporting electrolyte. In these experimental conditions, the obtained anodic current is close to zero, since poly-aniline can not be oxidized in absence of anions as counter ions. When the sample containing chloride reaches the amperometric detector this anion enables the poly-aniline oxidation giving rise to an anodic current proportional to chloride concentration. This FIA-amperometric method was successfully used for determine chloride content in six commercial ethanol fuel sample and the obtained results were in good agreement with those obtained by a comparative potentiometric method. All determinations were conducted without any sample pretreatment procedure which is a very attractive feature.

HPLC coupled to amperometric detection has been also used for quantification of organic species in ethanol fuel samples. The combination of separation ability of chromatography with high sensitivity of amperometric detection is a very useful strategy to analyze complex matrices such as biofuels, minimizing sample pretreatment requirements. Combination of LC with EC detection is a selective and sensitive tool for determination of a wide variety of organic compounds in several kinds of samples, including bioethanol and biodiesel. Trindade et al [143] have developed an analytical method based on HPLC with amperometric detection for the simultaneous quantification of SB-14 and solvent red 24 (SV-24) in ethanol fuel. The authors have observed that these dyes were better separated on a C18 column, using a mobile phase composed of acetonitrile and ammonium acetate (90:10, v/v). Detection was performed amperometrically using a glassy carbon electrode kept at +0.85V vs. $Ag/AgCl/KCl_{saturated}$. Recovery experiments were carried out in spiked commercial ethanol fuel samples which were analyzed after a fast filtration step in a 45 µm membrane. This analytical method has allowed the simultaneous detection of SB-14 and SV-24 in a very fast and reliable way since recovery percentages close to 100 % were obtained. HPLC coupled to amperometric detection was also used by Saczk et al [141] for quantification of carbonyl species in ethanol fuel samples. This chromatographic method was used to quantify several aldehydes and ketones after chemical derivatisation with 2,4-dinitrophenylhydrazine. The proposed method has enabled the simultaneous quantification of 5-hydroxymethylfurfural, 2-furfuraldehyde, butyraldehyde, acetone and methyl ethyl ketone in ethanol fuel in a fast and reliable way. The amperometric detection was performed by using a glassy carbon electrode kept at +1 V vs. $Ag/AgCl/KCl_{saturated}$. The chromatographic separation was carried out by using a C-18 column under isocratic conditions with a mobile phase containing a binary mixture of methanol/1.0 mmol L^{-1} $LiClO_{4(aq)}$ (80:20 v/v) and a flow-rate of 1.1 mL min^{-1}. The quantification of these carbonyl compounds is important to evaluate the ethanol oxidation process, since different aldehydes contents can be related to unsuitable condition of fermentation, transport and storage of fuel. HPLC with amperometric detection was also successfully used for quantification of acetaldehyde in ethanol fuel samples [142].

4. Conclusions and perspectives

We can observe that electroanalytical techniques are significantly contributing to bioethanol and biodiesel quality control by means of official analytical methods which are characterized by high simplicity and reliability associated with low cost and no need of specialized operators. We can also conclude that electroanalytical techniques will certainly continue contributing for bioethanol and biodiesel quality control by providing modern and efficient analytical methods which may be adopted by technical specifications in the future. It is interesting to observe that most of alternative electroanalytical-based methods are developed for determining species whose contents are not currently regulated. Therefore, the electroanalytical community is fulfilling its scientific role in anticipating solutions by developing analytical methods to quantify species which may become a future problem for the quality and commercialization of bioethanol and biodiesel.

We can also observe that the most sensitive electroanalytical methods employed for bioethanol and biodiesel analysis usually are based on CMEs. The development of this kind of electrode is a trend in electroanalytical chemistry since them present superior analytical performance comparatively to non-modified electrodes. Bioethanol and biodiesel are complex matrices, thus any analytical method employed to their analysis must be highly selective and sensitive. The use of CMEs coupled to modern electroanalytical techniques is able to satisfactorily respond to these requests. The use of analyte preconcentration coupled to high selective chemical modifiers has allowed measurements to be performed directly on biofuel or in slightly diluted biofuel samples. This brings a very important advance for biofuel analysis since it avoids time-consuming pretreatment procedures making the analytical method faster, simpler and more reliable. Thus researches on development of new highly selective chemical modifiers must be intensified in the next years.

Researches on electrodes that exploit the unique properties of nanomaterials such as nanoporous structures, nanoparticles, nanotubes, etc. have presented an impressive expansion lately. The use of nanostructured electrodes has brought impressive advances in stripping voltammetric analysis, since nanostructured modifiers present extremely attractive features such as high surface area, strong adsorptive nature resulting in lowered detection limits and high electrocatalytic activity. Thus nanostructured materials have an important role to play in the field of electroanalysis and also in biofuel electroanalysis. Therefore development and application of nanostructured electrodes for biofuel analysis will continue to expand.

Another major trend in electroanalysis is the miniaturization of electrodes and electrochemical cells because this makes electroanalytical methods highly portable allowing measures *in situ* and on line. Moreover, miniaturized electrochemical systems can be used as detectors in chromatographic methods combining the high sensitivity of electroanalytical techniques with the separation power of chromatography which is an excellent approach for analysis of complex samples such as biofuels.

We believe that the research on electroanalytical methods will continue to expand, focusing mainly on improving detection limits, selectivity and stability of CMEs. Research in the field of electrochemical sensors has not presented any sign of slowdown due mainly to the incorporation of nanomaterials into electrochemical sensors platforms. The stability of these modern electrochemical sensors has increased significantly which has enabled their use in very complex matrices. Thus, modern electrochemical sensors have potential to significantly contribute to quality control of biofuels in a very near future.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821