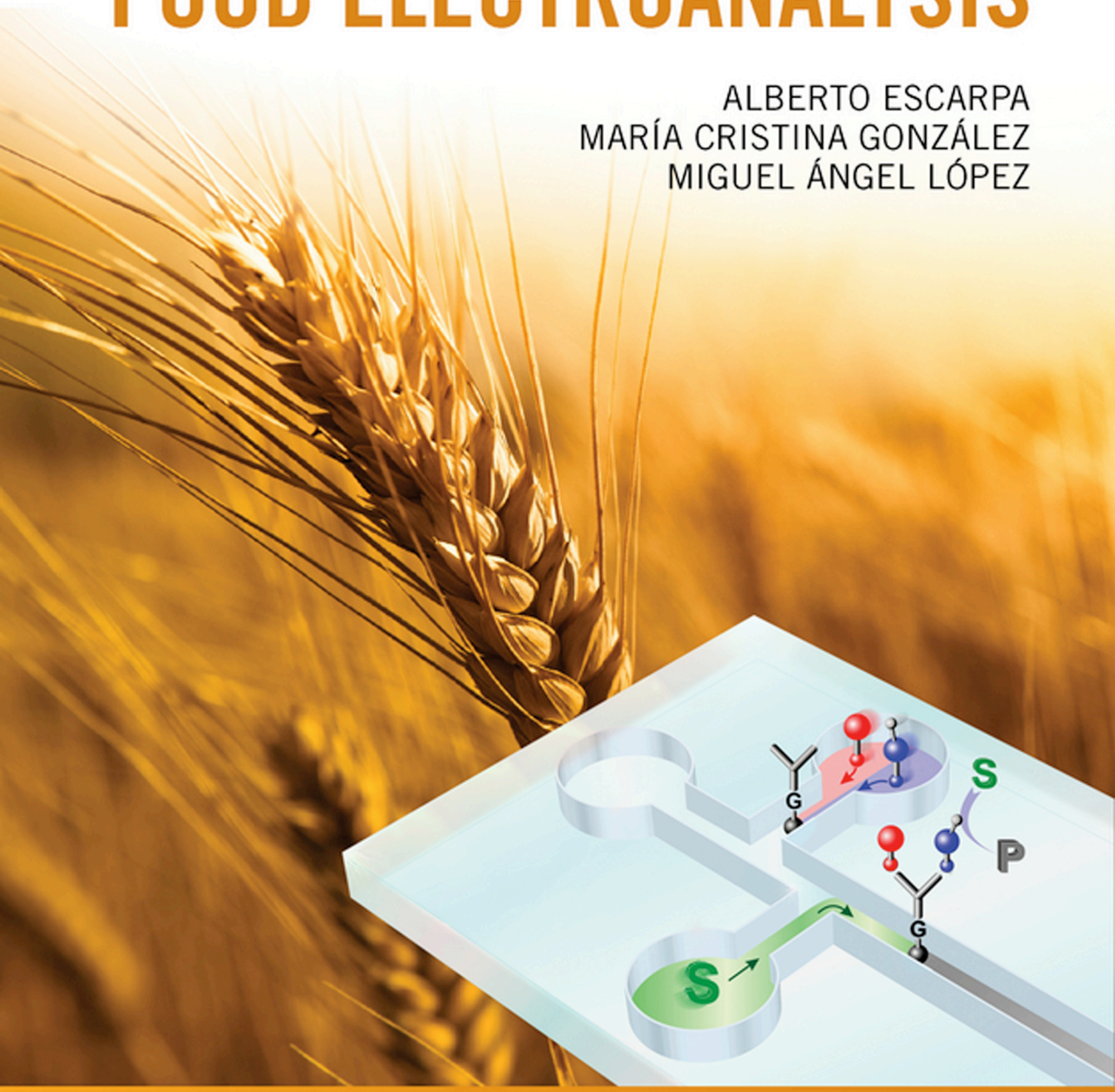
AGRICULTURAL AND FOOD ELECTROANALYSIS

ALBERTO ESCARPA
MARÍA CRISTINA GONZÁLEZ
MIGUEL ÁNGEL LÓPEZ



WILEY

Agricultural and Food Electroanalysis

Agricultural and Food Electroanalysis

Edited by

ALBERTO ESCARPA
MARÍA CRISTINA GONZÁLEZ
MIGUEL ÁNGEL LÓPEZ

Analytical Chemistry,
Physical Chemistry and Chemical Engineering Department,
University of Alcala,
Spain

WILEY

This edition first published 2015

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Library of Congress Cataloging-in-Publication Data

Agricultural and food electroanalysis / edited by Alberto Escarpa, Maria Cristina Gonzalez and Miguel Angel Lopez.
pages cm
Includes bibliographical references and index.
ISBN 978-1-119-96186-4 (cloth)
1. Food–Analysis. 2. Electrochemical analysis. I. Escarpa Miguel, Alberto, 1968- editor. II. Gonzalez Martin, Maria Cristina, editor. III. Lopez Gil, Miguel Angel, editor.
TX545.A39 2015
664′.07–dc23

2015016626

A catalogue record for this book is available from the British Library.

ISBN: 9781119961864
Cover Image: pawel.gaul/iStockphoto

Set in 10/12pt TimesLTStd by SPi Global, Chennai, India

1 2015

At the time we were editing this book, Professor Mascini passed away. Probably, one of the latest excellent contributions done in his vast successful career is found in this book. In memoriam, editors would like to dedicate him these words as proof of his valuable contribution to the field of electrochemical biosensors in food analysis.

Contents

List of Contributors

Lúcio Angnes, Instituto de Química, Universidade de São Paulo, Brazil

Alberto Sánchez Arribas, Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Craig E. Banks, School of Chemistry and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, UK

Mónica Moreno Barambio , Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Simona Benedetti, Dipartimento di Scienze per gli Alimenti, la nutrizione e l'ambiente Università degli Studi di Milano, Italy

Erika Bustos, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

Gang Chen, School of Pharmacy, Fudan University, China

Wendell K. T. Coltro, Institute of Chemistry, Federal University of Goias, Brazil

Stella M. Cosio, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy

Agustín Costa-García, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Alberto Escarpa, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Fabiana Silva Felix, Instituto de Química, Universidade de São Paulo, Brazil

M. Teresa Fernández-Abedul, Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Carlos D. Garcia, Department of Chemistry, The University of Texas at San Antonio, USA

Luis A. Godínez, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

María Cristina González, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Araceli González-Cortés, Departamento de Química Analítica, Facultad de Ciencias Química, Universidad Complutense de Madrid, Spain

M. Begoña González-García , Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain

Miguel A. López, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Spain

Saverio Mannino, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy

Juan Manríquez , Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro Sanfandila, México

Marco Mascini, Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Italy

Sandra Mendoza, Departamento de Investigación y Posgrado en Alimentos, Universidad Autónoma de Querétaro, México

Solomon Lemma Mengistu, Free University of Bolzano, Italy

Arben Merkoçi, Institució Catalana de Recerca i Estudis Avançats (ICREA), Spain; Nanobioelectronics and Biosensors Group, Universitat Autònoma de Barcelona, Spain

Jonathan P. Metters, School of Chemistry and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, UK

Maria F. Mora, Jet Propulsion Laboratory, California Institute of Technology, USA

Geza Nagy, General and Physical Chemistry, Faculty of Science, University of Pécs, Hungary

Lívia Nagy, Szentagothai Research Centre, University of Pécs, Hungary

Ilaria Palchetti, Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Italy

Briza Pérez-López, Nanobioelectronics and Biosensors Group, Universitat Autònoma de Barcelona, Spain; LEITAT Technological Center, Spain

José M. Pingarrón, Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Spain

Manuel Chicharro Santamaría, Departamento de Química Analítica y Análisis Instrumental, Universidad Autónoma de Madrid, Spain

Matteo Scampicchio, Free University of Bolzano, Italy

Joseph Wang, Departments of Nanoengineering, University of California, USA

Paloma Yáñez-Sedeño, Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Spain

Preface

This pioneer book *Agricultural and Food Electroanalysis* provides a description and rationale use of modern electroanalytical techniques, strategies, and approaches in the exciting field of agricultural and food analysis. Electrochemical techniques offer very valuable features such as very good sensitivity, tunable selectivity, low cost, simple use, inherent miniaturization, high compatibility with modern technologies required from microfabrication techniques to build "lab-on-a-chip" devices, high compatibility with surface modification employing biological reagents as well as exciting nanomaterials such as nanoparticles, nanotubes, and nanowires. Without any question, with the incursion of advanced approaches such as screen printed technology, biosensors, microchips, and nanotechnology, among others, electroanalysis is living a truly *Renaissance* and new frontiers have been clearly opened in the last years.

This book is divided in three parts and contains 16 chapters written by truly well-recognized experts in the field.

The first chapter, *Electroanalysis and Food Analysis,* has the important role to introduce the readers in the whole book where the adequacy of electroanalysis to agricultural and food analysis is exposed.

Following this initial introductory chapter, the book is structured in three parts. The first part discusses different *Electroanalytical Techniques in Batch and Continuous Systems* as highly remarkable tools in the agricultural and food field. In this sense, Chapter 2 deeply explores the sweep potential electroanalytical techniques, while Chapter 3 allows the readers to obtain fundamental information on voltammetric techniques coupled to flow systems which could proportionate faster analysis, reproducible results, high sensitivity, with additional advantages such as the requirement of less sample, and the use of simpler instrumentation.

Separation techniques coupled to electrochemical detectors are also studied in this section. Chapter 4 deals with the design and integration of electrochemical detectors within the HPLC separation system and their compatibility and compromise with the chromatographic conditions necessary to achieve the optimum resolution of the analytes in agricultural and food field. Chapter 5 introduces the key strategies in capillary electrophoresis using electrochemical detection for separating and detecting a variety of constituents in foods and agricultural products. That includes the commonly used separation modes of capillary electrophoresis, its coupling with electrochemical detection, and its application in agricultural and food analysis.

The largest part of the book, organized in the following nine chapters, is dedicated to the *Electrochemical Sensing in Food Analysis*. Chapter 6 introduces microelectrodes and

microelectrode arrays which can be used for both fundamental and applied electrochemistry. Different approaches to fabricate such transducers are critically overviewed with special emphasis on the requirement of sensors that can be used at the site of sampling, being cost-effective and reproducible. Besides, the importance of potentiometric sensors and electrochemical biosensing approaches is deeply studied in Chapters 7–11.

Electrochemical transducers combined with an enzyme as a biochemical component constitute the largest category of biosensors, thus becoming an important tool for the detection of highly concern analytes in agricultural and food monitoring. This matter is considered in Chapter 8. The design, chemical construction, and application in agricultural and food electroanalysis of the further most important biosensing approaches such as immunosensors and genosensors are studied in Chapter 9 and Chapter 10, respectively. Additionally, Chapter 11 discusses the recent trends that have led to powerful nanomaterial-based electrochemical biosensing devices and examines the related prospects and challenges suggesting considerable promise for diverse applications in the food and agricultural field.

The next two chapters of this book section address the novel micro- and nanotechnologies impact in the field. Electroanalysis on board of microfluidics and lab-on-a-chip platforms is studied in Chapter 12 and selected nanoelectrochemistry applications for food analysis are covered in Chapter 13. To conclude this part, Chapter 14 deals with the principles and food applications using electrochemical impedance spectroscopy.

The book finishes with two chapters configuring the third part regarding *Industrial Implications*: *Electroanalysis in Food Process Control* (Chapter 15) and *Instrumental Aspects of Food Analysis by Electrochemical Methods* (Chapter 16). Unlike traditional chemical analysis, performed in well-equipped laboratories with the aim to identify and quantify small amounts of analytes, the goal of process analytical chemistry is to supply quantitative and qualitative information about a chemical process that can be used not only to monitor and control the process, but also to optimize its efficient use of energy, time, and raw materials. In addition, it is possible to simultaneously minimize plant effluent release and to improve product quality. These important concepts adapted to food and agricultural electroanalysis are focused in Chapter 15. In contrast, despite the clear advantages associated with electrochemical detectors, the training of personnel and somehow the limited availability of commercial instruments has traditionally limited the development of electrochemical methods applied to agricultural and food-related samples. In the light of such considerations, Chapter 16 aims to provide a brief overview of the key instrumental aspects linked to agricultural and food electroanalysis.

In sum, and in editor's opinion, this valuable text offers a comprehensive vision about different electrochemical techniques following their basic principles, instrumentation, and main applications in the field of food and agricultural analysis. Also, editors hope that the critical and attractive vision of the book will help readers to get introduced in the exciting area of agricultural and food electroanalysis.

Finally, the editors would like to thank to all the authors for their excellent contributions to this pioneer book in the agricultural and food analysis field.

Alberto Escarpa, María Cristina González, and Miguel Ángel López
Editors

1

Electroanalysis and Food Analysis

Paloma Yáñez-Sedeño and José M. Pingarrón
Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, 28040, Madrid, Spain

1.1 Introduction and Adequacy of Electroanalysis for Food Analysis

Electroanalysis is a powerful analytical tool for food analysis. Since the early times of polarography and potentiometry until the current developments of chemical sensors, biosensors and lab-on-a-chip (LOC) devices involving electrochemical detection principles and many other electroanalytical methodologies have demonstrated their usefulness to accomplish the requirements imposed by the food industry for the analytical monitoring and control of raw materials and foodstuffs. This is particularly true in the last decades where impressing advances exhibited in automation, miniaturization, and easy handling of electroanalytical devices including both the corresponding instrumentation and the electrodes employed as electrochemical transducers, have led to user-friendly methods of analysis that are competitive against other well-established analytical techniques such as chromatography and spectroscopy. This, together with the inherent affordable costs of electroanalytical approaches and the superior sensitivity that can be achieved using modern voltammetric techniques or coupling with amplification response methodologies involving nanomaterials, makes modern electroanalysis a more than suitable strategy to face up to the increasingly demanding requirements of food industry to ensure food quality, control, and safety [1].

The development and innovation in food industry rely basically on the concepts of food safety and food quality. However, lately, products as foodstuffs supplemented with compounds such as omega-3 acids, vitamins, fiber, and so on, which confer them particular properties sought by specific strata of society, have burst into the modern food industry providing products with a high added value. Obviously, as the food chain is increasingly

Agricultural and Food Electroanalysis, First Edition.
Edited by Alberto Escarpa, María Cristina González and Miguel Ángel López.

complex, there is a high demand for the development of efficient traceability systems which are able to guarantee the firmness of the whole chain. These systems should possess high sensitivity, ability to be implemented rapidly, and permit automatic screening.

Food quality can be understood as a set of factors which are able to differentiate food products according to their organoleptic characteristics, composition, and functional properties. An increased regulatory action together with an increased consumer demand for information have led to the extensive labeling of major and minor constituents of the foodstuffs. The scientific evaluation of the food freshness is another important task concerning food quality assessment. A list of the most current compounds to be analyzed for food quality assessment can be found in [2]. Moreover, continuous monitoring of food industrial processes allow real-time detection of possible errors in the chain production as well as taking decisions to rectify such errors in an immediate manner. The assessment of food safety is the other key axe for the modern food industry. In general, one can speak about food contamination when dealing with harmful substances or microorganisms that are not intentionally added to the food. Contaminants may enter the food chain during growth, cultivation, or preparation, accumulate in food during storage, form in the food through the interaction of chemical components or may be concentrated from the natural components of the food [3]. However, chemicals are also added during food processing in the form of additives. At present, pathogen microorganisms, pesticides, animal-drug residues, and antimicrobial drug resistance are the main concerns for food safety. Food regulatory agencies have established control programs, such as the HACCP (Hazard Analysis Critical Control Point) program, to avoid the entering of these substances into the food chain [4].

Electroanalysis has played a relevant role in food quality and food safety assessment and, in the last few years, it is increasingly significant due to the combination of sensors and biosensors technology, even in a disposable manner, with efficient electrochemical transduction techniques allowing the implementation of rapid and reliable detection methods. To provide an overview of the state of art in the use of electrochemical techniques in the field of agricultural and food analysis, we discuss in this chapter some examples on the latest advances in this field, pointing out on relevant methodologies related to the measurement techniques, including the development of electrochemical sensors and biosensors for food components, and the use of nanostructured electrodes.

1.2 Methodologies Related to Measurement Techniques

1.2.1 Continuous Detection Methods

In general, the application of electrochemical techniques for the detection of analytes in a continuous mode has demonstrated to be able to improve the sensitivity and selectivity of well-established analytical methods. Electrochemical detection has shown to be appropriate to be combined with high-performance liquid chromatography (HPLC), flow injection analysis, capillary electrophoresis, or microfluidics-based methodologies. There are numerous examples regarding food analysis where the improvements achieved using electrochemical detection techniques can be illustrated. With respect to liquid chromatography, methods are still being developed for detecting healthy food components. Representative

examples are the simultaneous determination of hydroxy polymethoxy-flavones in citrus products and orange juice [5], and a very recent method for the determination of phenolics in olive oil [6]. In both cases, HPLC with coulometric detection at multichannel CoulArray detector was used which enabled a high sensitivity to be obtained. Moreover, methodologies developed for the detection of drugs and pesticide residues using electrochemical detection can be found in the recent literature. As examples, the efficient separation and sensitive determination of sulfonamides in shrimps using a monolithic column and amperometric detection at a boron-doped diamond electrode [7], and the detection of carbamate pesticides in fruits and vegetables using an acetylcholinesterase biosensor where the enzyme was immobilized on a polyaniline–carbon nanotubes composite electrode [8], can be cited.

Flow-injection methods with electrochemical detection continue to attract great attention in the field of food analysis due to the inherent simplicity of these approaches and the good analytical performance provided. A recent and interesting application involves a single-line flow injection system combined with multiple pulse amperometric detection with a boron-doped diamond electrode for the simultaneous determination of two pairs of food colorants: tartrazine (TT) and sunset yellow (SY) (TT–SY) or brilliant blue (BB) and SY (BB–SY) in sports drink beverages, gelatin, and powdered juice. A dual-potential waveform was applied to the electrode for both colorants in each pair to be determined with detection limits ranging between 0.80 and 3.5 μM [9]. Batch injection analysis (BIA) combined with electrochemical detection has also been applied in this field taking advantage of the versatility, reproducibility, high analytical frequency, sensitivity, portability, and sample size provided by this combination [10]. Using these systems, precise sample plugs are directly injected onto the working electrode surface which is immersed in a large-volume blank solution, and the electrochemical responses are recorded directly. For example, amperometric detection at a Prussian Blue-modified graphite-composite electrode was recently described for determining H_2O_2 in high- and low-fat milk samples [11]. In this method, an electronic micropipette injected 100-μl aliquots of 10-fold diluted samples directly onto the modified electrode immersed in the BIA cell (Figure 1.1). The detection limit was low (10 μM), and good recovery values were achieved for spiked samples.

Within this family of continuous methodologies, it is also important to mention the sequential injection lab-on-valve (SI-LOV) technique, which allows increasing sampling capacity and the automation of the analytical methods [12]. In a recent article, an SI-LOV system was used for the sensitive determination of hypoxanthine [13]. As one of the purine bases, hypoxanthine is produced during the degradation process of fresh meat and fish, so that the content of this compound can be envisaged as a valuable indicator of food freshness [14]. In the cited work, a Fe_3O_4/multiwalled carbon nanotubes (MWCNTs)/β-cyclodextrin (β-CD) (Fe_3O_4/MWCNTs/β-CD) modified electrode was employed to measure the electrochemical oxidation of hypoxanthine. A diagram of the SI-LOV system used is depicted in Figure 1.2. After aspiration of 500 μl phosphate buffer solution (PBS) into the holding coil, various microvolumes of carrier, air, sample solution, and PBS, were aspirated and transferred into electrochemical flow cell (EFC) for the analyte accumulation on the modified electrode at 0.1 V. Then, the stripping voltammogram of hypoxanthine was recorded. Under the optimized conditions, a linear dependence between log I_p *vs.* log [hypoxanthine] was found in the 5.0×10^{-8}–1.0×10^{-5} range, and the method was applied in determining hypoxanthine in meat samples.

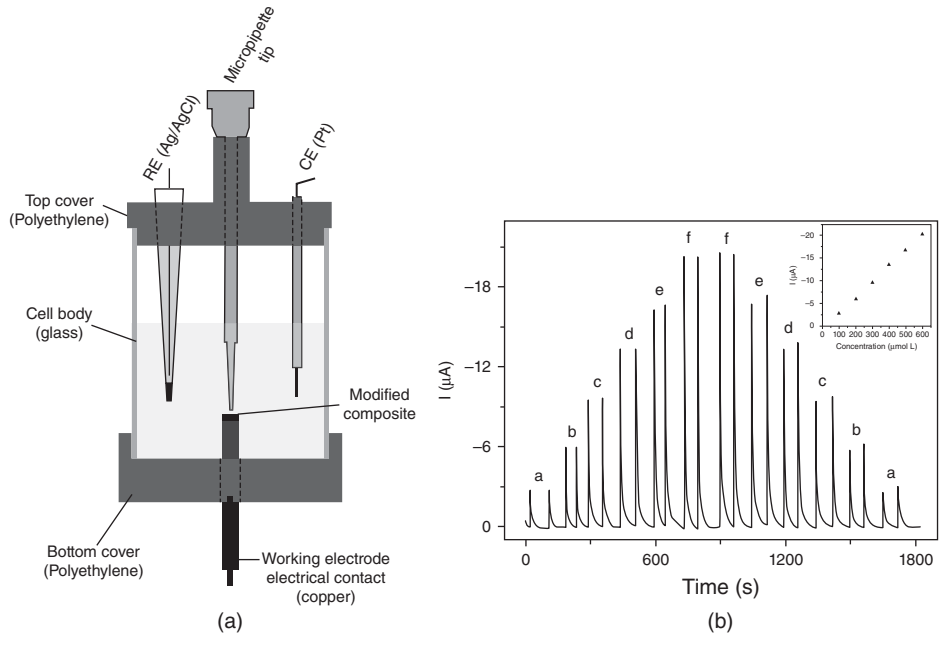


Figure 1.1 *(a) Schematic diagram of the batch injection cell containing the three-electrode system. (b) BIA amperometric responses of PB-modified graphite composite electrode for 100–600 μmol/l H_2O_2. Reproduced from Ref. [11] with permission from Elsevier*

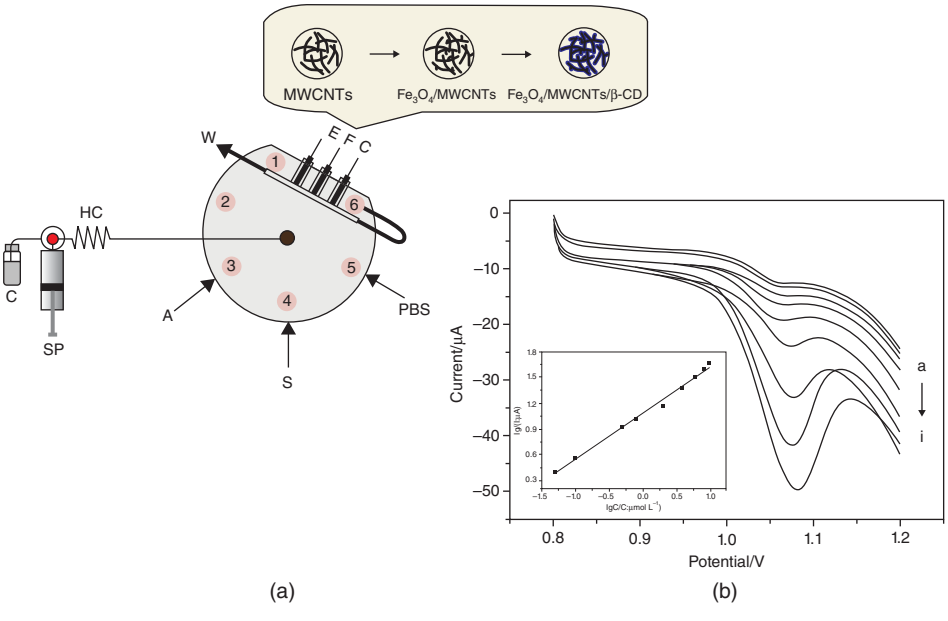


Figure 1.2 *(a) Schematic diagram of SI-LOV manifold for hypoxanthine analysis: C, carrier (H_2O); SP, syringe pump; HC, holding coil; W, waste; A, air; S, sample; PBS, phosphate buffer solution, EFC, electrochemical flow cell (internal volume 200 μl). (b) Stripping voltammograms for (a–i) 0.05–10 mmol/l hypoxanthine. Inset: log I_p vs. log hypoxanthine concentration calibration plot. Reproduced from Ref. [13] with permission from Elsevier*

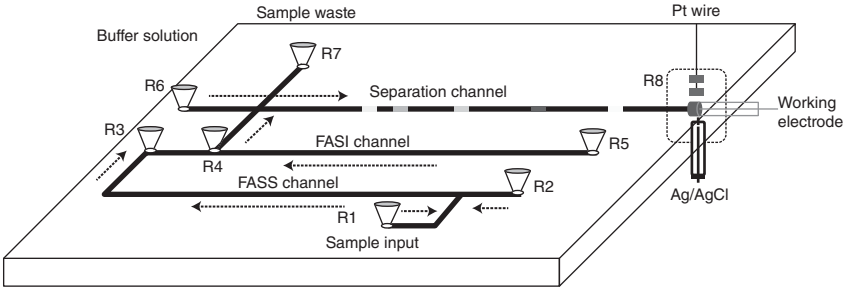


Figure 1.3 *Scheme of the microfluidic device for sulfonamides separation and detection. Reproduced from Ref. [16] with permission from Elsevier*

During the past 20 years, a great progress has been made in the development of miniaturized systems for chemical analysis. In this context, microfluidics has attracted special interest because of offering remarkable sensitivity, inherent miniaturization, low cost, portability, compatibility with mass fabrication, and on-site analysis. The manipulation of small amounts of fluids through microchannels can be combined with miniaturization technologies for developing "LOC" devices, which intend the integration of different steps involved in the analytical process. When a separation step is required, capillary electrophoresis (CE) has demonstrated to be very appropriate for miniaturized technology. A representative example of application of these systems to agricultural and food analysis is the determination of phenolic compounds (tyrosol, hydroxytyrosol, and oleuropein glucoside) in olive oil using glass microchip electrophoresis with end-channel amperometric detection at a 100-μm gold wire working electrode [15]. Other recent application makes use of a microfluidic device for the simultaneous detection of five sulfonamides in meat involving preconcentration of the analytes in the microfluidic device (Figure 1.3) followed by their electrokinetic separation and amperometric detection at Al_2O_3–gold nanoparticles (AuNPs)-modified carbon paste electrodes. A linear range between 0.01 and 2,025 pM, and detection limits between 0.91 and 2.21 fM were obtained [16].

Capillary electrophoresis with amperometric detection was also used for the determination of four electroactive preservatives (methylparaben, ethylparaben, propylparaben, and butylparaben) and, indirectly, two nonelectroactive preservatives (potassium sorbate and sodium lactate) in various types of foodstuffs [17]. Moreover, high-performance micellar electrokinetic capillary chromatography with amperometric detection (MECC–AD) has been also employed for the fast determination of melamine (2,4,6-triamino-s-triazine), which was occasionally used to increase the apparent protein content of milk products [18].

1.2.2 Stripping Analysis

Mercury film electrode has been widely used in stripping voltammetry for a long time owing to its excellent electrochemical behavior. However, the toxicity of mercury has led to the development and use of the environmentally friendly bismuth film electrode (BiFE). This electrode is characterized by the simple preparation process, large enough accessible potential window, high sensitivity, well-defined, and undistorted stripping signal, as well as good resolution of neighboring peaks. In addition, BiFE is less sensitive to the presence of

dissolved oxygen than MFE [19]. An interesting example of recent applications of stripping methodologies with BiFE in food analysis is the determination of azo-compounds used as dyes for food, beverages, and textile industry coloring, which represent a human hazard because their degradation products, including amines, are carcinogenic. A bismuth/poly(*p*-aminobenzene sulfonic acid) (*p*-ABSA) composite film-coated glassy carbon electrode (GCE) (Bi/poly(*p*-ABSA)/GCE), prepared by depositing bismuth on the poly(*p*-ABSA) modified electrode at −0.9 V, was used in this method. Azo-compounds such as 1-(2-pyridylazo)-2-naphthol (PAN), 4-(2-pyridylazo)-resorcinol (PAR), and azobenzene were determined by differential pulse voltammetry in orange and lemon beverages [20]. Other metallic films have also been employed for the preparation of modified electrodes to be applied in stripping methodologies for food analysis. For example, adsorptive stripping voltammetry (AdSV) with an *in situ* plated lead film GCE was used for the determination of vitamin B1 (thiamine) in juices. Thiamine was preconcentrated at −1.25 V and then electrochemically reduced by scanning the potential from −1.25 to −1.55 V using square wave voltammetry (SWV). A range of linearity between 0.0133 and 0.265 mg/l thiamine was reported with this methodology [21].

Despite the attempts to substitute mercury electrodes by other greener film electrodes, some recent applications involving Hg electrodes still appear in the literature. As an example, a method for the determination of the antibiotic ceftiofur (CF) in milk has been reported implying the adsorptive accumulation of the drug on a hanging mercury-drop electrode (HMDE) and reductive SWV. CF is a widely used broad-spectrum third-generation cephalosporin, which is approved for the treatment of infections in cattle, swine, sheep, goats, turkeys, and chickens. By application of the AdSV methodology, a linear calibration plot between 52.4 and 524 ng/ml, which allowed testing of the established tolerance level of 100 ng/ml for CF residues in bovine milk, was achieved. The method was applied to determine CF in spiked milk samples [22]. HMDE and SWV were also used for trace determination of azoxystrobin [methyl (E)-2-{2-[6-(2-cyanophenoxy)-pytimidin-4-yloxy]phenyl}-3-methoxyacrylate] and dimoxy-strobin [(E)-*o*-(2,5-dimethylphenoxymethyl)-2-methoxyimino-*N*-methyl phenylacetamide] in potatoes, grapes, and grape juice. These compounds are synthetic pesticides, the strobilurins, which are derived from the natural occurring β-methoxyacrylates. In this method, limits of quantification as low as 119 µg/l in grape juice and 45 µg/kg in potatoes and grapes were found using deposition potential and deposition time values of −300 mV and 30 s, respectively [23]. Differential pulse stripping voltammetry at an HMDE was also used for the simultaneous determination of tetracycline antibiotics in spiked animal feed and fresh fish muscle dosed with the drugs. The voltammograms from the drug mixture produced complex, overlapping profiles, and chemometrics methods were applied for calibration modeling. The analytical linear ranges were within 0.02–0.18 µg/ml and the corresponding limit of detections (LODs) were within 3–5 µg/l [24].

However, metal traces in foods were also determined by stripping analysis. For example, in a recent method, the sequential voltammetric determination of Hg(II) and Cu(II) at a gold electrode, and of Cu(II), Pb(II), Cd(II), Zn(II) at an HMDE by SW anodic stripping voltammetry in matrices involved in food chain as whole meal, wheat, and maize meal, was proposed. The supporting electrolyte was 0.01 mol/l EDTA-Na_2 + 0.06 mol/l NaCl + 2.0 mol/l $HClO_4$, and the analytical procedure was validated by the analysis of standard reference materials [25].

1.2.3 Potentiometry and Chronopotentiometry

Potentiometric detection using ion selective electrodes (ISEs) has had wide application in the field of food analysis since long time ago due to the fair selectivity, wide linear dynamic range, low cost, and automation ability of the derived methods [26]. Advances ISEs, developed in the last years, have also found application in this area. For example, solid-contact ion-selective platforms based on GCEs coated with electropolymerized polyaniline (PANI) and tetrasubstituted thiacalix[4]arene ionophores were reported for the discrimination of the brands of apple juices and herbal liqueurs. The samples were diluted and spiked with Fe^{3+}, and the variation of the signal from this ion, which is related to its reactivity with the organic ligands, was monitored. The method was also applied to the determination of antioxidants (ascorbic, malic, oxalic acids, hydroquinone, and quercetin) in the range from 5.0×10^{-6} to 1.0×10^{-2} M [27]. A potentiometric fumarate (FUM) ion selective electrode (Pt/Hg/Hg^{2+} FUM/graphite) has been recently developed for the determination of the acidulant additive fumaric acid in powdered foods such as gelatin, instant pudding, and ice cream. The achieved sensitivity was (-29.2 ± 0.6) mV/decade over a concentration range between 7.5×10^{-7} and 1.0×10^{-2} M [26]. A graphite carbon electrode coupled with a flow system was also used for the potentiometric determination of citrate in fruit juices and an isotonic drink. The fundamentals of this procedure involve the ion-exchange adsorption process of citrate on the electrode surface and the subsequent potential change. The electrode exhibits a linear response, with a slope of -29.0 ± 1.0 mV/decade, in a 0.07–7.0 mM concentration range with an LOD of 3.0 μM [28].

Chronopotentiometric analytical methods have also found application in the field of food analysis. In this technique, the oxidation or reduction of species at a constant current is carried out, and the transition time is measured as the quantitative characteristic [29]. In this context, recent chronopotentiometric methods have been reported for histamine determination in foodstuffs. One of these methods was based on the oxidation of the amine at a planar gold disc electrode in the presence of electrogenerated chlorine which facilitates charge transfer between the analyte and the electrode surface. Well-defined signals were observed at +1.15 V in hydrochloric acid medium, giving rise to a linear calibration plot in the 2–100 mg/l concentration range with an LOD of 0.27 mg/l histamine. The method was applied to the determination of histamine in fermented sausages [30]. The same authors used a mercury film electrode to develop a chronopotentiometric method for histamine in cheese [29].

1.2.4 Electronic Tongues

Electronic tongues are multisensor systems with marked mix-response, capable of giving a wide and complete response toward the analyzed species [31]. Advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate analysis, able to extract meaningful data from the complex readings, are usually needed in their applications [32]. Electronic tongues involving arrays of electrodes suitable for voltammetric experiments have been applied in food quality studies, namely in wines [33], milk [34], and fruit juices [35]. An illustrative recent example is the development of an electronic tongue that combined non-noble metals (Ni, Co, and Cu) and noble metals (Au, Pt, Rh, Ir, and Ag) for the determination of chloride, nitrate, and nitrite in minced meat. A rational

design of the waveform used by the electronic tongue and multivariate analysis including cross validation and partial least square (PLS) to build suitable management and prediction models for the analysis were reported [36]. Another electronic tongue has been developed for the simultaneous determination of the ethanol acetaldehyde, diacetyl, lactic acid, acetic acid, and citric acid content in probiotic fermented milk. The sensor array comprised of seven nonspecific, cross-sensitive sensors coupled with a reference Ag/AgCl electrode. Samples of plain, strawberry, apple-pear, and forest-fruit flavored probiotic fermented milk were analyzed and the results were used for the development of neural network models for rapid estimation of the aroma compounds content in probiotic fermented milk [37].

Monitoring of biotechnological processes, including fermentation, by determination of physicochemical parameters allows the suitable control of the process ongoing including the detection of possible relevant perturbations. A sensor array composed of potentiometric and voltammetric sensors (Figure 1.4a) was proposed as an efficient tool to control the production process of beer. The sensor array consisted of 10 miniaturized ion-selective electrodes and silicon based three-electrode voltammetric transducers. The obtained results were processed using PLSs and PLS-DA (partial least squares-discriminant analysis). The samples originated from batch of homemade beer fermentation and from two stages of the process: fermentation reaction and maturation of beer [38]. Also, a bioelectronic tongue has been constructed for the estimation of the polyphenol content in wine. The approach involved an array of four voltammetric enzyme biosensors (Figure 1.4b) using epoxy–graphite composites and a chemometric processing tool, which is able to interpret the chemical signals and extract meaningful data from the complex readings. One blank

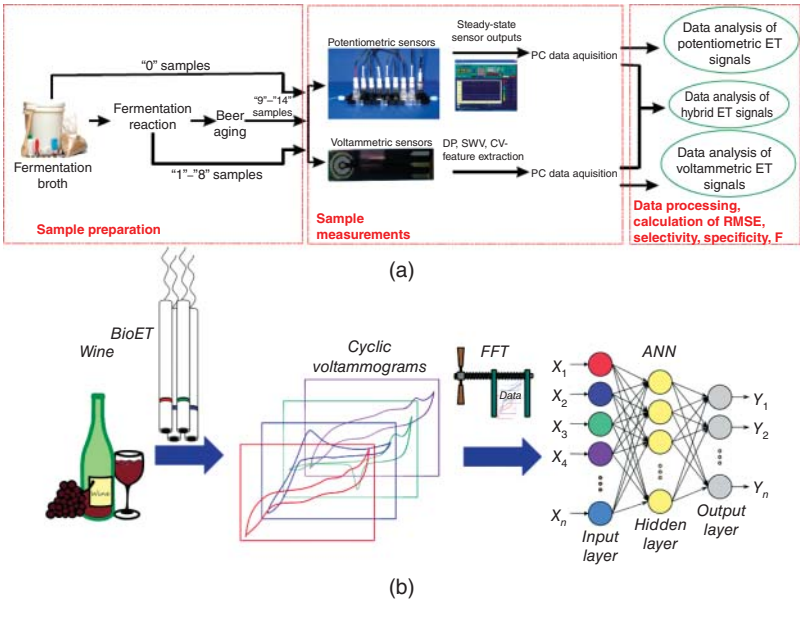


Figure 1.4 *Schemes of the experimental setups of: (a) a hybrid electronic tongue for the control of the beer production process. Reproduced from Ref. [38] with permission from Elsevier. (b) A bioelectronic tongue for quantification of polyphenols in wine. Reproduced from Ref. [31] with permission from Elsevier*

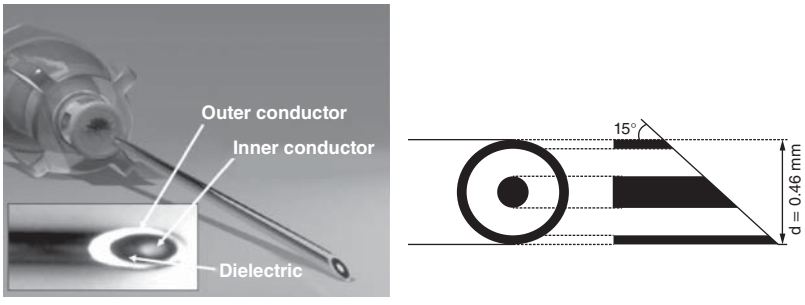


Figure 1.5 *Coaxial needle electrode used for the analysis of food samples by impedance measurements. Reproduced from Ref. [42] with permission from Elsevier*

electrode and the other three bulk modified with tyrosinase and laccase on one side, and copper nanoparticles on the other side, were used in order to incorporate differentiated or catalytic response to different polyphenols present in wine. The obtained voltammetric responses were preprocessed employing the fast Fourier transform (FFT) whereas the obtained coefficients fed an artificial neural network (ANN) model that accomplished the quantification of total polyphenol content [31].

1.2.5 Impedance Spectroscopy

Various applications of impedance spectroscopy have been described in food engineering, such as monitorization of yogurt processing [39], salt and moisture measurement in salmon fillets [40], and testing of meat quality [41]. A relevant example is the construction of a low-cost and nondestructive system to evaluate the salt levels in food based on a punctual measurement of the impedance in the samples. A coaxial electrode, consisted of an isolated wire inserted into a hollow needle, facilitating the placement inside the food sample, was used (Figure 1.5). Furthermore, the impedance modulus and phase values obtained for each frequency were processed using PLS in order to estimate and predict the salt content in minced pork meat [42].

1.3 Electrochemical Sensors and Biosensors for Food Components

Food analysis is a major application area of electrochemical sensors and biosensors. One of the key aspects driving their dramatic development in recent years has been, among others, the use of nanomaterials. As it is outlined below, nanoscience and nanotechnology has strongly influenced the design and construction of recent electrochemical sensors and biosensors paving the way for nanostructured electrode surfaces which are able to improve the quality of the electrochemical response and allowing the efficient immobilization of biomolecules.

1.3.1 Molecularly Imprinted Electrodes

Apart from ISEs, there are very few examples of electrochemical sensors, which are able to provide specific responses to particular analytes without the support of biological

recognition elements. Among these, devices using molecularly imprinting polymers (MIPs) have found a certain degree of popularity lately. As it is well known, molecularly imprinting is as a synthetic methodology intended to create tailor-made binding sites with the memory of shape, size, and functional groups toward the template molecules [43]. Various designs of molecularly imprinted electrodes, mainly based on the preparation of electropolymerized imprinted films have been applied to food analysis. For example, a computer-assisted design of MIPs was used to screen functional monomers and solvents for cyanazyne, a triazine herbicide, as the template molecule. The obtained MIP, embedded in a carbon paste electrode, functioned as a preconcentrator agent and selective recognition element for cyanazine determination by using cathodic stripping voltammetry. The efficiency of the prepared MIP electrode for the extraction of the CZ in tomato, onion, lettuce, and rice, spiked with cyanazyne, was demonstrated [44]. A molecularly imprinted electrochemical impedimetric sensor was constructed for the selective determination of melamine. The sensor consisted of a gold electrode modified by electrochemical polymerization of 2-mercaptobenzimidazole (2-MBI) using cyclic voltammetry (CV) in the presence of the template molecule melamine. A linear response was obtained between 1.0×10^{-8} and 5.0×10^{-5} M, with a detection limit of 3.0×10^{-9} M and the method was applied to the analysis of spiked liquid, powder milk and yogurt samples [45] (Figure 1.6).

1.3.2 Enzyme Biosensors

Twenty years ago, Professor Saverio Mannino and Professor Joseph Wang, published a review article entitled "Electrochemical Methods for Food and Drink Analysis." In this

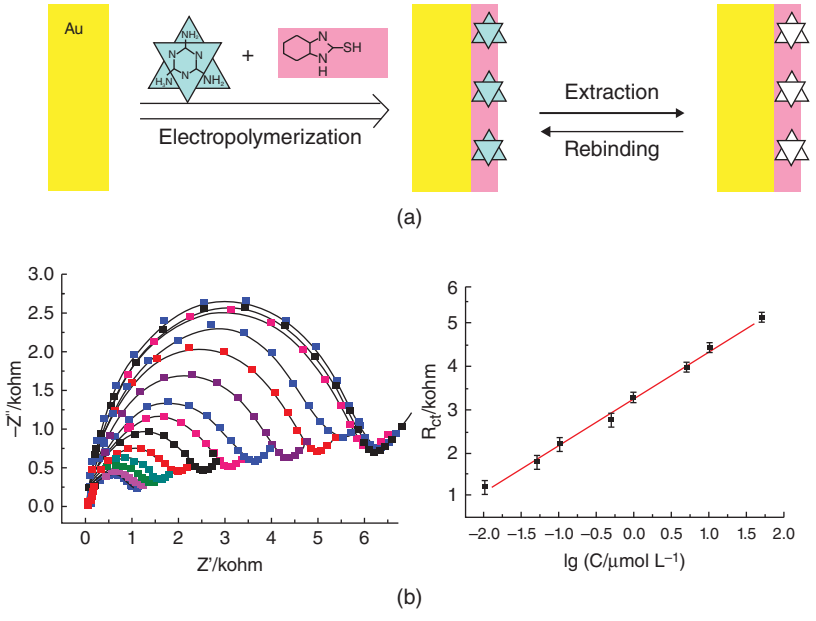


Figure 1.6 *(a) Scheme of the sensor preparation and (b) electrochemical impedance responses for 0–0.001 M melamine and calibration plot. Reproduced from Ref. [45] with permission from Elsevier*

review they had stated that: "Many target analytes in the food and beverage industries are primary substrates of oxidase or dehydrogenase enzymes, and can thus be easily sensed with amperometric transducers. Other biological entities should play a growing role in the future" [46]. In fact, at present, a great variety of enzyme electrode-based biosensors are available to detect many analytes of interest in the food industry. Coupling of the inherent specificity of enzymes with the advantageous properties of electrochemical transducers, particularly, their high sensitivity, low cost, and simplicity of the methodologies still remains the main attraction for the application of these analytical tools in food industries. The most studied analytes include glucose, fructose, and other carbohydrates, cholesterol, and ethanol. However, recently, enzymatic biosensors have been designed and applied to the determination of other less common target analytes in food samples. For example, an amperometric and impedimetric biosensor for detecting trimethylamine (TMA), which represents a good parameter for estimating fish freshness, has been developed. A conducting polypyrrole substituted with ferrocenyl was used to immobilize flavin-containing monooxygenase 3 (FMO3) enzyme which catalyzed the monooxygenation TMA to trimethylamine N oxide (TMO). The evaluation of fish freshness was realized by measuring the extract sample from horse-mackerel fish [47]. An electrochemical microdevice was also fabricated for on-site determination of rapid freshness of rice by monitoring the activity of peroxidase using hydrogen peroxide as enzyme substrate and hydroquinone as redox mediator for amperometric detection. Peroxidase is one of the key enzymes involved in the elimination of reactive oxygen species and is directly related to the deterioration of rice grains. The rate of current change originating from benzoquinone, a reduction product of the mediated enzymatic reaction, on a gold electrode, depended on the rice freshness, reflecting the remaining activity of peroxide which is more rapid with fresh rice (Figure 1.7). The device with multiple sensing sites demonstrated the possibility to identify a mixture of grains [48].

As it was mentioned above, the use of nanomaterials has attracted a great attention in the development of a new generation of biosensors. The unique physical, chemical, and electronic properties of nanomaterials make possible the intimate attachment of enzymes providing enhanced electroanalytical performance. Among the most widely used nanomaterials, gold nanoparticles have been found to be particularly useful due to the easy synthesis and functionalization, high chemical stability, low inherent toxicity (biocompatibility), and tunable optical and electronic properties [49]. Many interesting examples of biosensors involving the use of gold nanostructures as an appropriate support to immobilize enzymes can be found in the recent literature. For example, an electrochemical biosensor for the determination of total cholesterol was fabricated by co-immobilizing three enzymes, cholesterol oxidase (ChOx), cholesterol esterase (ChE), and horseradish peroxidase (HRP), on nanoporous gold networks which were directly grown on a titanium substrate (Ti/NPAu/ChOx–HRP–ChE). The developed biosensor possessed high selectivity and high sensitivity ($29.33\,\mu A\,mM^{-1}\,cm^{-2}$), as well as a wide linear range up to $300\,mg\,dl^{-1}$. The fabricated biosensor was validated by analyzing margarine, butter, and fish oil samples [49].

Nanostructured enzyme electrochemical biosensors were also recently prepared for the determination of polyphenols, the most abundant antioxidants present in diet. One configuration involved immobilization of laccase onto nickel nanoparticles (NiNPs) decorated-carboxylated multiwalled carbon nanotubes (cMWCNTs)/PANI and was applied to the

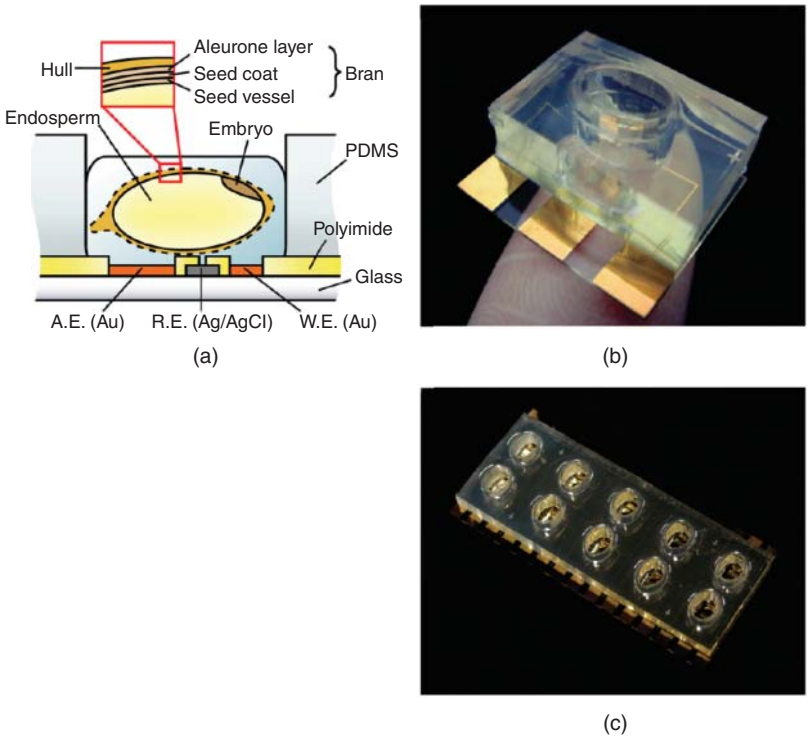


Figure 1.7 *(a) Cross-section of the electrochemical microdevice for the determination of rice freshness, and a rice grain. (b and c) Images of the devices for 1 and 10 rice grains. Reproduced from Ref. [48] with permission from Elsevier*

determination of total polyphenols in fruit The enzyme electrode could be used up to 200 times over a period of four months when it was stored at 4°C [50]. Another example relies on laccase immobilization onto nanocomposites of silver nanoparticles and zinc oxide nanoparticles which were electrochemically deposited onto gold electrodes. The method was applied to the determination of total phenolic compounds in wine and a linear range for guaiacol was reported in the 0.1–500 µM concentration range [51].

1.3.3 Affinity Biosensors

The applications of immunosensors and genosensors in the field of food analysis are diverse, the more recent encompassing allergen detection and monitoring of species associated with celiac disease, toxins, microorganisms, genetically modified organisms (GMOs), and pesticide or drug residues, among others. The detection of okadaic acid (OA) is a challenging and important issue for shellfish industries worldwide. This compound is a lipophilic marine biotoxin produced by Dinophysis and Prorocentrum dinoflagellates. OA intoxication is considered as the most of concern diarrhetic shellfish poisoning (DSP) for human health [52]. An electrochemical immunosensor was developed recently for the determination of OA based on an indirect competitive immunoassay format under

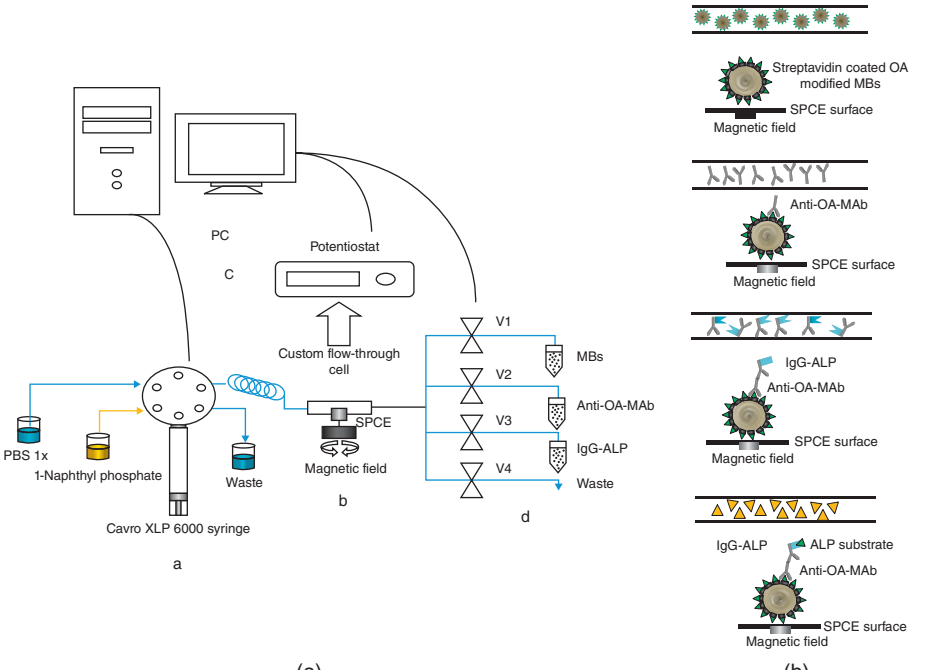


Figure 1.8 *Schematic diagram of the apparatus used for continuous flow automated okadaic acid analysis. A: (a) Syringe pump; (b) electromagnetic device; (c) recorder device; and (d) selection valves. B: Schematic representation of the rationale relying the flow-based immunoassay. Reproduced from Ref. [52] with permission from Elsevier*

automated flow conditions. The biosensor was fabricated by injecting OA modified magnetic beads onto screen printed carbon electrode in the flow system (Figure 1.8). The OA present in the sample competed with the immobilized OA to bind with anti-okadaic antibody, and the secondary alkaline phosphatase-labeled antibody was used to perform electrochemical detection. The method was applied to the analysis of mussels [52].

A disposable amperometric immunosensor was also developed for sensitive detection of chlorpyrifos-methyl, a persistent insecticide widely used in farming and horticulture. The device was fabricated by modification of the screen-printed carbon electrodes with nanocomposites made by skillful doping of bovine serum albumin conjugated chlorpyrifos-methyl and platinum colloid into silica sol–gel. The immobilization of BSA–Ag conjugates on the nanocomposite retained their immunoactivities, which allowed the immobilized BSA–Ag to effectively capture unbound Ab-HRP in the detection solution. A linear response to chlorpyrifos-methyl concentration ranging from 0.4 to 20 ng/ml was reported and good results were obtained for the detection of the insecticide in treated soil and grape samples [53].

Gliadin, a constituent of the cereal protein gluten, is responsible for the intolerance generated in celiac disease. Control of gliadin contents in gluten-free foods is needed to prevent the inappropriate immune response in patients. Various immunosensors have been reported in the literature with this objective. As an example, an electrochemical magneto

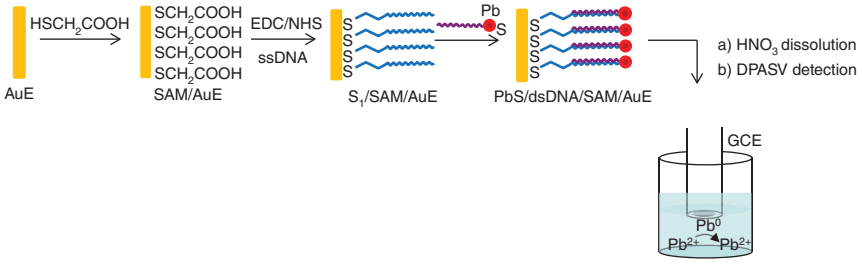


Figure 1.9 *Scheme of the preparation of an electrochemical DNA biosensor using PbS nanoparticle labels*

immunosensor was described relying on the immobilization of gliadin onto tosyl-activated magnetic beads. A direct competitive format providing a detection limit in the order of $\mu g\, l^{-1}$ was used to determine gliadin in spiked gluten-free foodstuffs (skimmed milk and beer) obtaining excellent recoveries [54].

Regarding DNA sensors, a few examples can be considered to demonstrate their usefulness in the field of food analysis. One concerns microorganisms as target analytes and involved the development of a biosensor based on target-induced aptamer displacement for direct detection of *Escherichia coli O111*. The aptamer was immobilized on a gold electrode through hybridization with a capture probe anchored to the electrode surface *via* Au-thiol binding. In the presence of *E. coli O111*, the aptamer was dissociated from the capture probe–aptamer duplex due to the stronger interaction between the aptamer and *E. coli O111*. The consequent single-strand capture probe could be hybridized with biotinylated detection probe and tagged with streptavidin–alkaline phosphatase, producing sensitive enzyme-catalyzed electrochemical responses. Using this strategy, a detection limit of 305 CFU ml^{-1} in milk within 3.5 h was obtained [55].

Electrochemical DNA biosensors are also appropriate for the detection of GMOs due to their well-known advantages of simplicity, low cost, and ease of use. In a recent article, a DNA biosensor was developed for determining the CaMV 35S sequence, which was derived from the cauliflower mosaic virus from *Agrobacterium tumefaciens,* and is often used as an insert and indicator of GMOs. The DNA biosensor design involved the synthesis of mercaptoacetic acid-modified lead sulfide nanoparticles which were used as specific ssDNA sequence labels. The target ssDNA was covalently fixed on a SAM-modified gold electrode (Figure 1.9), and the hybridization process was monitored by dissolution of PbS nanoparticles anchored on the hybrids by oxidation with nitric acid and determination of lead ion by differential pulse anodic stripping voltammetry. The CaMV 35S target sequence was detected with a detection limit of 4.38×10^{-12} mol l^{-1} [56].

1.4 Nanomaterials for Electrochemical Analysis of Food

We have already commented that the application of nanotechnology has highly improved the designs and the way of preparing platforms for electrochemical analysis. In addition to the examples already described, other representative configurations should be mentioned in this section because of their relevance, as they are representative examples of the great usefulness of nanomaterials in the field of food analysis.

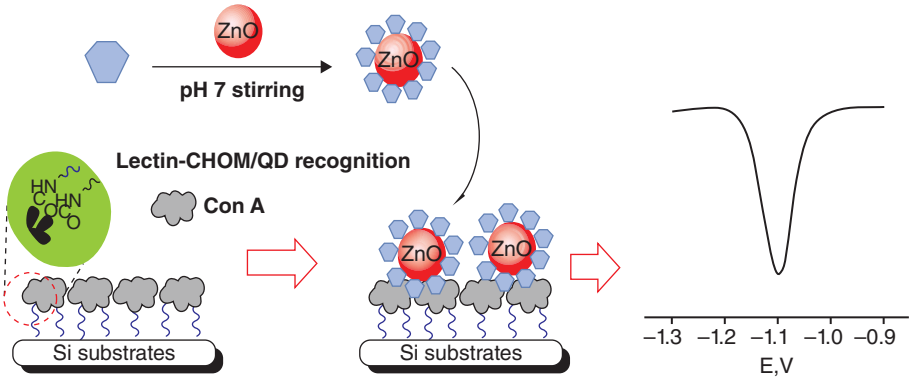


Figure 1.10 *Scheme of the preparation of bioconjugates for the electrochemical detection of chicken ovomucoid allergen (CHOM). Reproduced from Ref. [57] with permission from Elsevier*

For example, the quantum dot (QD) labeled methodology, which has been profusely applied in the construction of optical sensors, has been also used for the preparation of bioconjugates that can be electrochemically detected. The high surface-to-volume ratio of QDs combined with stripping voltammetry led to signal amplification allowing highly sensitive detection. An illustrative example is the development of a concanavalin A-based sensor for the direct electrochemical detection of chicken ovomucoid allergen. The biomimetic and high isoelectric point ZnO QDs material was used for self-assembling of the analyte through electrostatic interaction between negatively charged protein and positively charged QDs surface (Figure 1.10). Concanavalin A was employed as a recognition element for ZnO QDs-labeled allergen, and the detection process was monitored by stripping SWV of captured QDs [57].

Glucose monitoring has great interests in food industry. Numerous glucose oxidase-based enzyme electrochemical biosensors have been developed for this purpose. However, the immobilization of the enzyme on the surface of transducers can complicate the preparation of biosensors and affect their final performance. Moreover, instability of glucose oxidase humpers the use of these biosensors for continuous monitoring in production or transformation processes such as fermentation. Nonenzymatic glucose sensors, which are able to determine glucose in a direct manner, constitute an attractive alternative to overcome these problems. Direct electrochemical oxidation of glucose is readily noticeable onto Ni, NiO, or $Ni(OH)_2^-$ modified electrodes due to the redox couple of $Ni(OH)_2$/NiOOH formed on the electrode surface on alkaline medium. For example, nano NiO-modified carbon paste electrodes were employed to determine glucose in real juice samples [58].

Lately, graphene-based electrochemical platforms are being increasingly used for numerous applications. Although there are still few examples in the area of food analysis, it can be mentioned that some interesting biosensor configurations involving the nanomaterial modification by covalent functionalization have appeared in the recent literature. For example, a label-free voltammetric immunosensor for the sensitive detection of β-lactoglobulin in milk using graphene-modified screen-printed electrodes was described. Graphene was derivatized by electrografting of 4-nitrophenyl diazonium cations followed by electrochemical

reduction and activation with glutaraldehyde for the covalent attachment of β-lactoglobulin antibodies. The analyte determination was performed by monitoring the decrease in the differential pulse voltammetric current from $[Fe(CN)_6]^{3-/4-}$. A detection limit of 0.85 pg/ml and a dynamic range from 1 pg/ml to 100 ng/ml β-lactoglobulin were achieved, and the method was validated by application to the analysis of various foodstuffs containing milk derivatives and comparison with commercial ELISA protocols.

1.5 Future Trends

Throughout this chapter, different recent examples on application of electroanalytical techniques and methodologies in the field of food analysis have been commented. They are nice examples of the great potentiality of modern electroanalysis in this important technological and industrial area not only for the detection and quantification of different species at very low concentrations, taking advantage of the great sensitivity and low limits of detection that contemporary electroanalytical techniques allow, but also for the monitoring and control of food components with high precision and accuracy. These inherent excellent performance of electroanalysis together with the low cost of the required instrumentation and maintenance, constitute synergistic strengths that it is expected to contribute for offering new, affordable, and attractive alternative methods suitable to be implemented in quality control laboratories in the near future. Furthermore, it is most likely that new applications of biosensors using both enzymatic designs and affinity designs will appear and the continued use of new generation nanomaterials will contribute in providing amplified and enhanced electroanalytical responses as well as improving the immobilization ability of the modified electrode surfaces. Regarding relevant target analytes, it is foreseen that new electroanalytical methods will be devoted to the detection of allergens, drug residues, and toxins, as well as to the determination of components of interest that are present in functional foods and their relationship with deficiency diseases or obesity, among others.

Acknowledgments

Financial support from the Ministerio de Ciencia e Innovación (Projects CTQ2012-35041 and CTQ2012-34238), and Comunidad de Madrid S2013/MT-3029 (NANOAVAnSENS) is gratefully acknowledged.

References

1. Escarpa, A. (2012) Food electroanalysis: sense and simplicity. *Chem. Rec.*, **12**, 72–91.
2. Serra, B., Reviejo, A.J. and Pingarrón, J.M. (2007) in Electrochemical Sensor Analysis, Wilson & Wilson's Comprehensive Analytical Chemistry, Chapter 13 (eds S. Alegret and A. Merkoçi), Elsevier, Amsterdam, pp. 257–258.
3. Pividori, M.I. and Alegret, S. (2007) in Electrochemical Sensor Analysis, Wilson & Wilson's Comprehensive Analytical Chemistry, Chapter 22 (eds S. Alegret and A. Merkoçi), Elsevier, Amsterdam, p. 467.

4. Rooney, R. and Wall, P.G. (2003) Food safety, in Encyclopedia of Food Science and Nutrition (eds B. Caballero, L. Trugo and P.M. Finglas), Academic Press, New York, pp. 27–30.
5. Dong, P., Qiu, P., Zhu, Y. *et al.* (2010) Simultaneous determination of four 5-hydroxy polymethoxyflavones by reversed-phase high performance liquid chromatography with electrochemical detection. *J. Chromatogr. A*, **1217**, 642–647.
6. Bayram, B., Ozcelik, B., Schultheiss, G. *et al.* (2013) A validated method for the determination of selected phenolics in olive oil using high-performance liquid chromatography with coulometric electrochemical detection and a fused-core column. *Food Chem.*, **138**, 1663–1669.
7. Sangjarusvichai, H., Dungchaia, W., Siangproh, W. and Chailapakula, O. (2009) Rapid separation and highly sensitive detection methodology for sulfonamides in shrimp using a monolithic column coupled with BDD amperometric detection. *Talanta*, **79**, 1036–1041.
8. Cesarino, I., Moraes, F.C., Lanza, M.R.V. and Machado, S.A.S. (2012) Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilised on a composite of polyaniline-carbon nanotubes. *Food Chem.*, **135**, 873–879.
9. Medeiros, R.A., Lourencao, B.C., Rocha-Filho, R.C. and Fatibello-Filho, O. (2012) Flow injection simultaneous determination of synthetic colorants in food using multiple pulse amperometric detection with a boron-doped diamond electrode. *Talanta*, **99**, 883–889.
10. Quintino, M.S.M. and Angnes, L. (2004) Batch injection analysis: an almost unexplored powerful tool. *Electroanalysis*, **16**, 513–523.
11. Silva, R.A.B., Montes, R.H.O., Richter, E.M. and Muñoz, R.A.A. (2012) Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection. *Food Chem.*, **133**, 200–204.
12. de Castro, M.D.L., Ruiz-Jimenez, J. and Perez-Serradilla, J.A. (2008) Lab-on-valve: A useful tool in biochemical analysis. *TrAC, Trends Anal. Chem.*, **27**, 118–126.
13. Wang, Y., Wang, L., Tian, T. *et al.* (2012) A highly sensitive and automated method for the determination of hypoxanthine based on lab-on-valve approach using Fe_3O_4/MWCNTs/β-CD modified electrode. *Talanta*, **99**, 840–845.
14. Hernández-Cázares, A.S., Aristoy, M.C. and Toldrá, F. (2010) Hypoxanthine-based enzymatic sensor for determination of pork meat freshness. *Food Chem.*, **123**, 949–954.
15. Godoy-Caballero, M.P., Acedo-Valenzuela, M.I., Galeano-Díaz, T. *et al.* (2012) Microchip electrophoresis with amperometric detection for a novel determination of phenolic compounds in olive oil. *Analyst*, **137**, 5153–5160.
16. Won, S.Y., Chandra, P., Hee, T.S. and Shim, Y.B. (2013) Simultaneous detection of antibacterial sulfonamides in a microfluidic device with amperometry. *Biosens. Bioelectron.*, **39**, 204–209.
17. Wang, W., Wang, Y., Zhang, J. *et al.* (2010) Simultaneous determination of electroactive and non-electroactive food preservatives by novel capillary electrophoresis with amperometric detection. *Anal. Chim. Acta*, **678**, 39–43.

18. Wang, J., Jiang, L., Chu, Q. and Ye, J. (2010) Residue analysis of melamine in milk products by micellar electrokinetic capillary chromatography with amperometric detection. *Food Chem.*, **121**, 215–219.
19. Wang, J. (2005) Stripping analysis at bismuth electrodes: a review. *Electroanalysis*, **17**, 1341–1346.
20. Xie, X.Y., Luo, H.Q. and Li, N.B. (2010) Determination of azo compounds by differential pulse voltammetry at a bismuth/poly(*p*-aminobenzene sulfonic acid) film electrode and application for detection in food stuffs. *J. Electroanal. Chem.*, **639**, 175–180.
21. Tyszczuk-Rotko, K. (2012) New voltammetric procedure for determination of thiamine in commercially available juices and pharmaceutical formulation using a lead film electrode. *Food Chem.*, **134**, 1239–1243.
22. Barbosa, A.M.J., de Araújo, T.A., Trinidade, M.A.G. and Ferreira, V.S. (2011) A simple method for electroanalytical determination of ceftiofur in UHT milk samples using square-wave voltammetry technique. *J. Appl. Electrochem.*, **41**, 249–255.
23. Pacheco, W.F., Doyle, A., Duarte, D.R.A. *et al.* (2010) Square-wave adsorptive stripping voltammetry for trace determination of dimoxystrobin and azoxystrobin in potatoes and grapes. *Food Anal. Methods*, **3**, 205–210.
24. Ni, Y., Li, S. and Kokot, S. (2011) Simultaneous voltammetric analysis of tetracycline antibiotics in foods. *Food Chem.*, **124**, 1157–1163.
25. Locatelli, C. and Melucci, D. (2012) Voltammetric determination of ultra-trace total mercury and toxic metals in meals. *Food Chem.*, **130**, 460–466.
26. Oppermann Santini, A., Redigolo Pezza, H. and Pezza, L. (2012) Development of a sensitive potentiometric sensor for determination of fumaric acid in powdered food products. *Food Chem.*, **134**, 483–487.
27. Evtugyn, G.A., Belyakova, S.V., Shamagsumova, R.V. *et al.* (2010) Discrimination of apple juice and herbal liqueur brands with solid-state electrodes covered with polyaniline and thiacalixarenes. *Talanta*, **82**, 613–619.
28. Araujo, C.L., Melo, E.I. and Coelho, N.M.M. (2011) Potentiometric detection of citrate in beverages using a graphite carbon electrode. *Talanta*, **84**, 1169–1173.
29. Švarc-Gajić, J. and Stojanović, Z. (2011) Determination of histamine in cheese by chronopotentiometry on a thin film mercury electrode. *Food Chem.*, **124**, 1172–1176.
30. Stojanovic, Z.S. and Svarc-Gajic, J.V. (2011) A simple and rapid method for histamine determination in fermented sausages by mediated chronopotentiometry. *Food Control*, **22**, 2013–2019.
31. Cetó, X., Céspedes, F. and del Valle, M. (2012) Bioelectronic tongue for the quantification of total polyphenol content in wine. *Talanta*, **99**, 544–551.
32. del Valle, M. (2010) Electronic tongues employing electrochemical sensors. *Electroanalysis*, **22**, 1539–1555.
33. Pigani, L., Foca, G., Ulrici, A. *et al.* (2009) Classification of red wines by chemometric analysis of voltammetric signals from PEDOT-modified electrodes. *Anal. Chim. Acta*, **643**, 67–73.
34. Dias, L.A., Peres, A.M., Veloso, A.C.A. *et al.* (2009) An electronic tongue taste evaluation: identification of goat milk adulteration with bovine milk. *Sens. Actuators, B*, **136**, 209–217.

35. Gutés, A., Ibáñez, A.B., del Valle, M. and Céspedes, F. (2006) Automated SIA e-Tongue employing a voltammetric biosensor array for the simultaneous determination of glucose and ascorbic acid. *Electroanalysis*, **18**, 82–88.
36. Campos, I., Masot, R., Alcañiz, M. *et al.* (2010) Accurate concentration determination of anions nitrate, nitrite and chloride in minced meat using a voltammetric electronic tongue. *Sens. Actuators, B*, **149**, 71–78.
37. Hruskar, M., Major, N., Krpan, M. and Vahcic, N. (2010) Simultaneous determination of fermented milk aroma compounds by a potentiometric sensor array. *Talanta*, **82**, 1292–1297.
38. Kutyła-Olesiuk, A., Zaborowski, M., Prokaryn, P. and Ciosek, P. (2012) Monitoring of beer fermentation based on hybrid electronic tongue. *Bioelectrochemistry*, **87**, 104–113.
39. Kitamura, Y., Toyoda, K. and Park, B. (2000) Electric impedance spectroscopy for yogurt processing. *Food Sci. Technol. Res.*, **6**, 310–313.
40. Chevalier, D., Ossart, F. and Ghommids, C. (2006) Development of a non-destructive salt and moisture measurement method in salmon (Salmo salar) fillets using impedance technology. *Food Control*, **17**, 342–347.
41. Damez, J.L., Clerjon, S., Abouelkaram, S. and Lepetit, J. (2008) Beef meat electrical impedance spectroscopy and anisotropy sensing for non-invasive early assessment of meat ageing. *J. Food Eng.*, **85**, 116–122.
42. Masot, R., Alcañiz, M., Fuentes, A. *et al.* (2010) Design of a low-cost non-destructive system for punctual measurements of salt levels in food products using impedance spectroscopy. *Sens. Actuators, A*, **158**, 217–223.
43. Chen, L., Xu, S. and Li, J. (2011) Recent advances in molecular imprinting technology: current status, challenges and highlighted applications. *Chem. Soc. Rev.*, **40**, 2922–2942.
44. Gholivand, M.B., Torkashvand, M. and Malekzadeh, G. (2012) Fabrication of an electrochemical sensor based on computationally designed molecularly imprinted polymers for determination of cyanazine in food samples. *Anal. Chim. Acta*, **713**, 36–44.
45. Wu, B., Wang, Z., Zhao, D. and Lu, X. (2012) A novel molecularly imprinted impedimetric sensor for melamine determination. *Talanta*, **101**, 374–381.
46. Mannino, S. and Wang, J. (1992) Electrochemical methods for food and drink analysis. *Electroanalysis*, **4**, 835–840.
47. Bourigua, S., El Ichi, S., Youssoufi, H.K. *et al.* (2011) Electrochemical sensing of trimethylamine based on polypyrrole–flavin-containing monooxygenase (FMO3) and ferrocene as redox probe for evaluation of fish freshness. *Biosens. Bioelectron.*, **28**, 105–111.
48. Koyachi, E., Kojima, K., Qiu, X. *et al.* (2013) Electrochemical microdevice for on-site determination of rice freshness. *Biosens. Bioelectron.*, **42**, 640–645.
49. Ahmadalinezhad, A. and Chen, A. (2011) High-performance electrochemical biosensor for the detection of total cholesterol. *Biosens. Bioelectron.*, **26**, 4508–4513.
50. Chawla, S., Rawal, R., Sharma, S. and Pundir, C.S. (2012) An amperometric biosensor based on laccase immobilized onto nickel nanoparticles/carboxylated multiwalled carbon nanotubes/polyaniline modified gold electrode for determination of phenolic content in fruit juices. *Biochem. Eng. J.*, **68**, 76–84.

51. Chawla, S., Rawal, R., Kumar, D. and Pundir, C.S. (2012) Amperometric determination of total phenolic content in wine by laccase immobilized onto silver nanoparticles/zinc oxide nanoparticles modified gold electrode. *Anal. Biochem.*, **430**, 16–23.
52. Dominguez, R.B., Hayat, A., Sassolas, A. *et al.* (2012) Automated flow-through amperometric immunosensor for highly sensitive and on-line detection of okadaic acid in mussel sample. *Talanta*, **99**, 232–237.
53. Wei, W., Zong, X., Wang, X. *et al.* (2012) A disposable amperometric immunosensor for chlorpyrifos-methyl based on immunogen/platinum doped silica sol–gel film modified screen-printed carbon electrode. *Food Chem.*, **135**, 888–892.
54. Laube, T., Kergaravat, S.V., Fabiano, S.N. *et al.* (2011) Magneto immunosensor for gliadin detection in gluten-free foodstuff: towards food safety for celiac patients. *Biosens. Bioelectron.*, **27**, 46–52.
55. Luo, C., Lei, Y., Yan, L. *et al.* (2012) A rapid and sensitive aptamer-based electrochemical biosensor for direct detection of *Escherichia coli O111*. *Electroanalysis*, **24**, 1186–1191.
56. Sun, W., Zhong, J., Qin, P. and Jiao, K. (2008) Electrochemical biosensor for the detection of cauliflower mosaic virus 35 S gene sequences using lead sulfide nanoparticles as oligonucleotide labels. *Anal. Biochem.*, **377**, 115–119.
57. Yang, C., Gu, B., Xu, C. and Xu, X. (2011) Self-assembled ZnO quantum dot bioconjugates for direct electrochemical determination of allergen. *J. Electroanal. Chem.*, **660**, 97–100.
58. Mu, Y., Jia, D., He, Y. *et al.* (2011) Nano nickel oxide modified non-enzymatic glucose sensors with enhanced sensitivity through an electrochemical process strategy at high potential. *Biosens. Bioelectron.*, **26**, 2948–2952.

Part I

Electroanalytical Techniques in Batch and Continuous Systems in Food Analysis

2

Voltammetric Techniques

Sandra Mendoza[1], *Erika Bustos*[2], *Juan Manríquez*[2] *and Luis A. Godínez*[2]

[1]*Departamento de Investigación y Posgrado en Alimentos, Universidad Autónoma de Querétaro, Cerro de las Campanas s/n, Querétaro, QRO, 76010, México*

[2]*Department of Science, Centro de Investigación y Desarrollo Tecnológico en Electroquímica, S. C., Parque Tecnológico Querétaro s/n, Sanfandila, Pedro Escobedo, QRO 76703, México*

2.1 Introduction

Measuring the quality, safety, nutrition, and stability of food products is a concern of analytical chemistry. Although thin layer chromatography (TLC)-densitometry, high performance liquid chromatography coupled to mass spectrometry (HPLC-MS), gas chromatography coupled to mass spectrometry (GC-MS), capillary electrophoresis, and spectroscopy among others, have proven to be reliable methods for the identification and quantification of several components in food products, there is still a need for fast, accurate, reproducible, and sensitive analytical techniques. In this regard, conventional methods suffer from some disadvantages, such as high cost, long analysis time, sample pretreatment requirements, and in some cases, low sensitivity and selectivity. Compared with other methods, electrochemical methods are characterized by simplicity, high sensitivity, good stability, low-cost instrumentation, and small sample requirements. In this chapter, a brief review of sweep potential electrochemical techniques as well as some relevant literature concerning identification and quantification of food components will be addressed.

2.2 An Overview of Sweep Potential Electrochemical Techniques

Potential sweep electrochemical techniques involve the interfacial potential scan of a selected potential window and the corresponding measurement of the current responses,

Agricultural and Food Electroanalysis, First Edition.
Edited by Alberto Escarpa, María Cristina González and Miguel Ángel López.

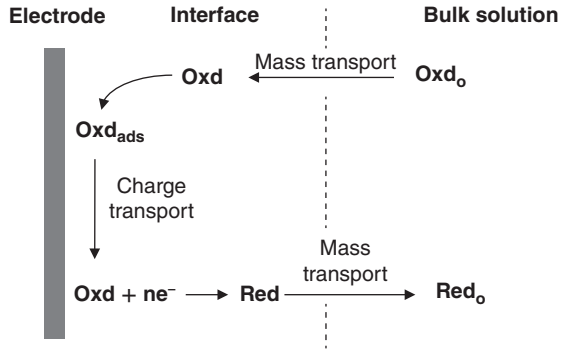


Figure 2.1 *Electrochemical reaction events*

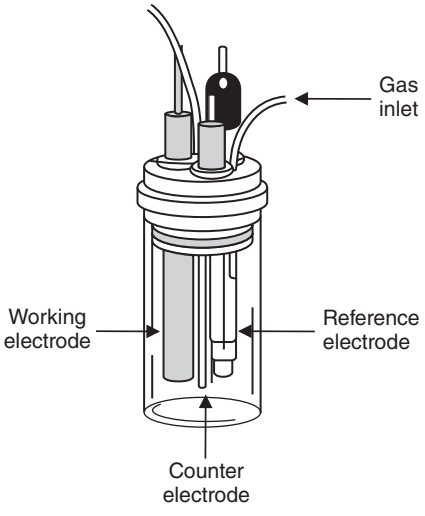


Figure 2.2 *Scheme of a typical three-electrode cell used in voltammetry techniques, where the gas inlet is used for bubbling the electrolyte solution with an inert gas*

which are produced from electron transfer events between the electroactive species and the working-electrode material (Figure 2.1). Since this phenomenon involves oxidation or reduction reactions of the electroactive species, the measured currents can be considered proportional to the concentrations.

The instrumentation for carrying out potential sweep techniques typically involves a three-electrode cell (Figure 2.2), where working- and counter electrodes are immersed in an electrolyte solution containing the electroactive species. A reference electrode however, is used to maintain a controlled potential at the working electrode by means of a potentiostat. It is also important to point out that the electrolytic solutions are commonly bubbled with an inert gas (such as nitrogen or argon) in order to remove oxygen, which could react in the potential window under study and interfere with the relevant current responses [1–3].

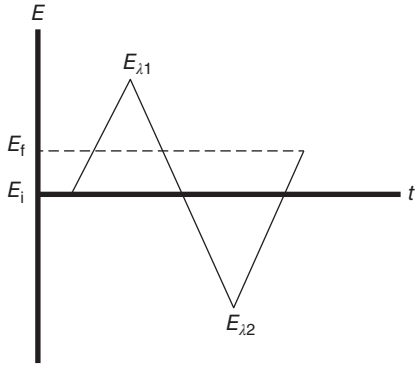


Figure 2.3 *CV potential vs. time perturbation wave-form*

2.2.1 Linear Sweep Voltammetry/Cyclic Voltammetry

Linear sweep voltammetry (LSV) and cyclic voltammetry (CV) are the most commonly used potential sweep techniques. The perturbation signal of the working electrode (Figure 2.3) is based on a linear potential wave-form, where the potential E is changed with the time (t) in a linear fashion. The rate of potential change is known as the *scan rate* v ($= dE/dt$), which typically varies between 1 mVs^{-1} and 1 Vs^{-1} [3, 4].

In LSV, the explored potential region is scanned from the initial potential (E_i) to the final potential (E_f). CV however, is an extension of LSV since the direction of the potential scan is reversed at the end of the first scan (which then becomes the switching potential, $E_{\lambda 1}$), and the final potential becomes the starting potential. In some experiments, the perturbation can be stopped at $E_f = E_i$, or instead, the potential can be scanned past this potential to a predefined second switching potential value ($E_{\lambda 2}$), where the direction of the potential scan is reversed again. The potential can therefore be cycled between the two switching potentials for as many cycles as needed before the experiment is ended at the E_f.

The current response, I, in voltammetry experiments corresponds to the addition of charging (I_c) and faradaic (I_f) current contributions, which in turn are associated with the interfacial charging and the electron transfer events that take place at the electrode surface (see Equation 2.1),

$$I = I_c + I_F = vC_d + I_F \tag{2.1}$$

while $I_c \propto v$ (the interfacial capacitance, C_d, is the proportionality constant), $I_F \propto C_s v^{1/2}$, where C_s corresponds to the concentration of the electroactive species in the electrolytic solution. In practical terms however, the capacitive contribution in Equation 2.1 is eliminated by subtraction of the blank response (electrolytic solution without electroactive species). In this way, detection limits for LSV and CV have been reported to be around 10^{-5} M [5].

The most common response of an LSV experiment is a peak-shaped curve (Figure 2.4) from which it can be observed that the current starts to increase at the activation potential (E_a). This potential value corresponds to the energy onset at which the electrochemical reaction takes place. As the potential moves from E_a, a continuous depletion of electroactive species near the electrode surface occurs, reaching a point at the peak potential (E_p), in

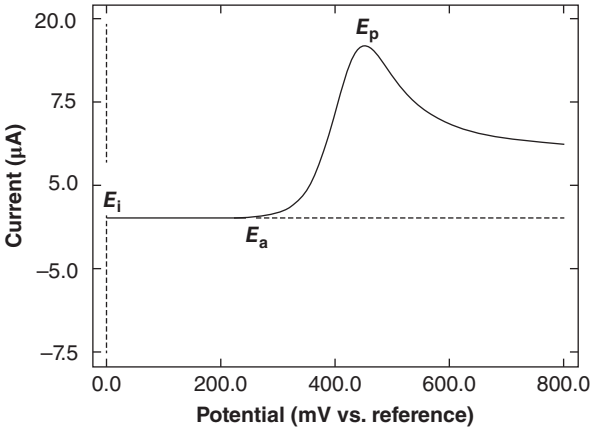


Figure 2.4 *Typical LSV experimental response*

which the electrochemically reactive species has been completely transformed. Beyond this potential value, the current response is characterized by a current decreasing curve that reflects the mass transport control of the electrochemical reaction.

During the reverse potential sweep in CV experiments on the other hand, the product of the initial oxidation or reduction of the electroactive species is complementary reduced or oxidized (Figure 2.5). In this context, E_{pa} and E_{pc} correspond to the anodic and cathodic potential peaks, respectively [1, 6, 7]. It is important to note that for reactions characterized by fast electron transfer events relative to the time-scale of the potential sweep rate (*reversible reactions,* where the difference $E_{pa} - E_{pc}$ is independent of v), the formal thermodynamic electrode potential $E^{\circ\prime}$ can be readily estimated using Equation 2.2.

$$E^{\circ\prime} = \frac{1}{2}\,(E_{pa} + E_{pc}) \tag{2.2}$$

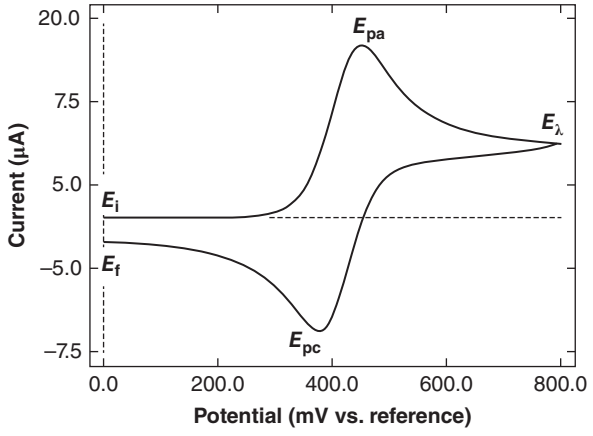


Figure 2.5 *Typical CV experimental response*

2.2.2 Pulse Voltammetry Techniques

Pulse voltammetry techniques are characterized by a succession of potential steps. During the sequential potential steps, the rates of current decay of the capacitive (I_c) and the faradaic currents (I_F) are essentially different (specifically, while I_c in Equation 2.1 decays exponentially with time, I_F decreases as a function of $t^{-\frac{1}{2}}$, characteristic of a diffusion-controlled electrochemical reaction). In this way, the rate of decay of I_c is significantly faster than that of I_F, and thus I_c is negligible at a time of $\sim 5R_uC_d$ after the potential step is imposed (where R_uC_d is the time constant, τ_{cell}, for the electrochemical cell having values from microseconds to milliseconds, and R_u is the uncompensated resistance between reference and working electrodes). Consequently, I_F is the main contribution to the measured current I when its value is measured at the end of a potential step. The detection limits of these techniques therefore fall around 10^{-7} M making them suitable for quantitative analysis.

The most important parameters for pulse voltammetric techniques are defined as follows. *Pulse amplitude* is the height of the potential pulse, which may or may not be constant depending on the technique. *Pulse width* is the duration of the potential pulse. *Sample period* is the time of the pulse at which the current is measured. A number of different pulse techniques are available in commercial potentiostats, which essentially differ in their potential step wave-forms and the number of sampling points [1].

2.2.3 Normal Pulse Voltammetry

The potential wave-form for normal pulse voltammetry (NPV) is shown in Figure 2.6. This typical perturbation consists of a series of pulses of increasing potential amplitude that ends at the initial potential value. The sigmoidal shape response for NPV technique (Figure 2.7) resembles that of a typical I–E curve obtained in a classical polarography experiment [2, 7].

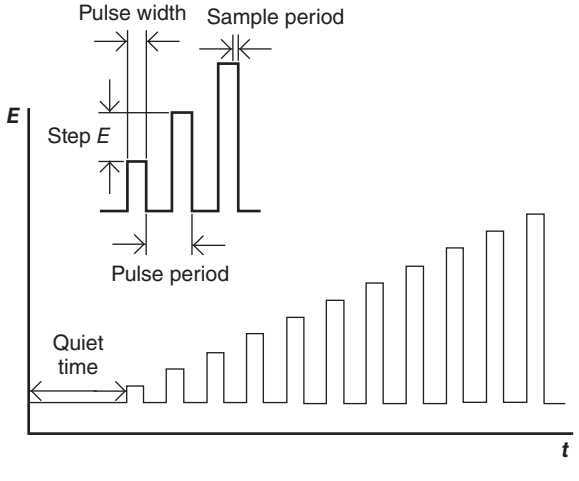


Figure 2.6 *Potential perturbation wave-form in a NPV experiment*

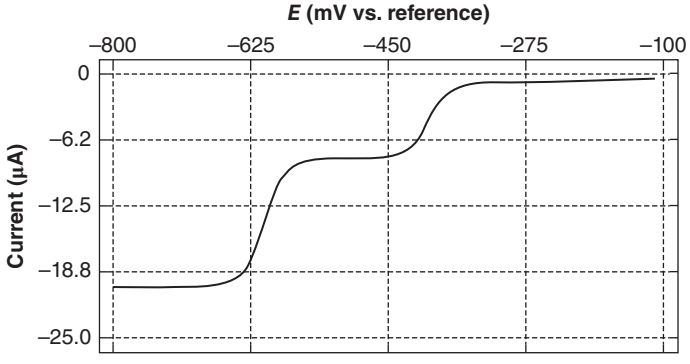


Figure 2.7 *Sigmoidal-type response obtained from a NPV experiment*

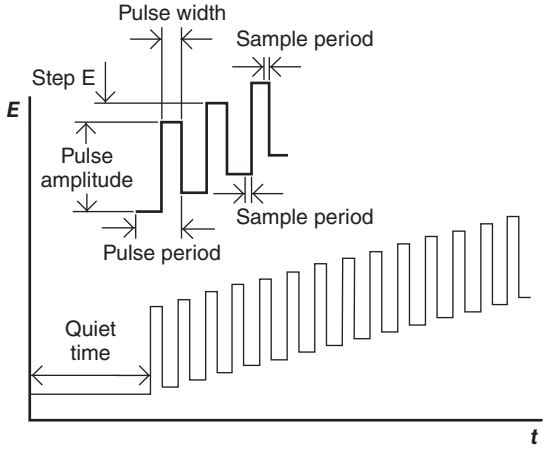


Figure 2.8 *Potential wave form used as perturbation in a DPV experiment*

2.2.4 Differential Pulse Voltammetry

The potential wave-form for differential pulse voltammetry (DPV) is shown in Figure 2.8. The perturbation consists of a series of pulses having constant amplitude, superimposed at the same time upon a staircase wave-form. In contrast with NPV, the current is sampled twice at each pulse period (at the beginning and at the end of the pulse). The difference between these two current values is recorded and displayed as function of the applied potential, *E*, as shown in Figure 2.9, where it is observed that the current function response is characterized by symmetric peaks [4, 8].

2.2.5 Square Wave Voltammetry

The excitation potential wave-form for square wave voltammetry (SWV) is shown in Figure 2.10. The perturbation consists of a square wave having constant amplitude,

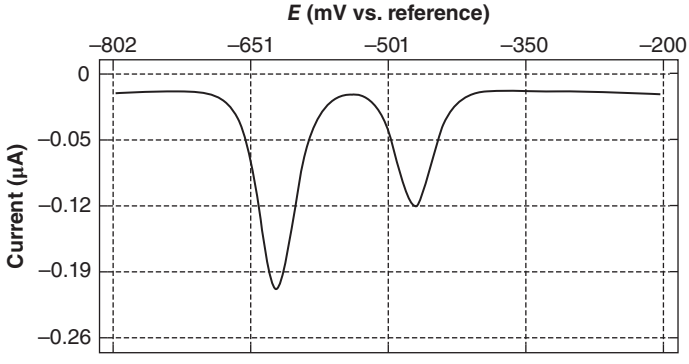


Figure 2.9 *Response from a DPV experiment showing symmetric peaks*

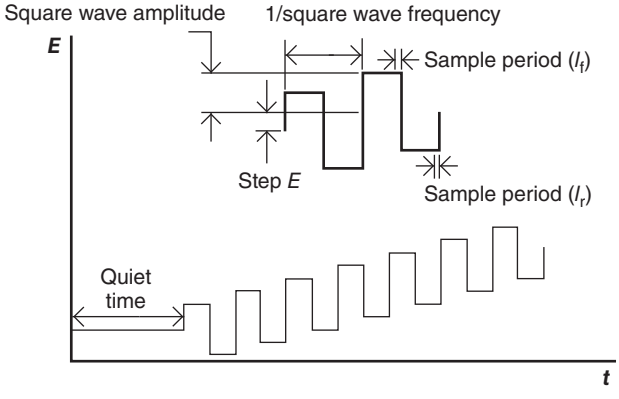


Figure 2.10 *Perturbation potential wave-form in an SWV experiment*

superimposed to a staircase wave form. The current is measured at the end of each forward half-cycle (I_f) and at the end of each reverse half-cycle (I_r). The difference between both current values ($I_f - I_r$) is displayed as a function of the applied potential E as shown in Figure 2.11. Therefore, the effect of the charging current is notably decreased in SWV since any residual charging current is completely removed [1, 4].

2.2.6 Stripping Voltammetry

The first stage in stripping electroanalytical methods is the accumulation of the electroactive substance either on the surface or in the bulk of a liquid electrode (mercury electrodes). The second stage consists of electrode polarization, obtaining cathodic or anodic voltammograms (stripping voltammetry (SV)) that give information on the nature and concentration of electroactive analytes [7].

In this context, anodic stripping voltammetry (ASV) is a very sensitive electrochemical technique mainly used for the analysis of trace concentrations of metallic species in solution, although detection of some organic species has also been carried out. Detection limits