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Voltammetric/amperometric screening of compounds of pharmacological interest

Abstract: Voltammetric and amperometric methods for screening compounds of pharmacological interest are reviewed on the basis of the types of electrodes and analytical strategies. The scope of conventional voltammetric and amperometric methods has been considerably expanded in the last years due to the development of a plethora of modified electrodes, in particular, those involving nanocomposites. The application of solid state voltammetric techniques for pharmacological screening has been also reviewed.

Keywords: amperometry; electrochemical screening; pharmacology; voltammetry.

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Introduction

Screening of compounds of pharmacological interest is an obvious analytical target in the fields of pharmacology and biomedical chemistry. Electrochemical methods have been extensively used in such fields for determining different types of pharmacologically active compounds. They have also been used for targeting biochemical activity, namely, antioxidant capacity (Andlauer and Héritier 2011), antitumoral activity (Richardson et al. 2006), electrochemical cofactor regeneration for electroenzymatic reduction of redox mediators (Gajdzik et al. 2012), enzymatic activity (Abdellaoui et al. 2013), among many others. Correlation between electrochemical properties and bioactive properties was reviewed by De Abreu et al. (2002) and this correlation has experienced a significant expansion during the last years due to the implementation of high-throughput methodologies derived from the application of the principles of combinatorial chemistry to electrochemical screening (Ozkan 2010, Pinkzewska et al. 2012). Other increasing growing fields are those based on electro-optical assays (or screens) (Zhao et al. 2009) and bioelectrochemical sensors, these last were constituted with a biological recognition component directly connected to an electrochemical transducer (De Souza Gil and Rodrigues de Melo 2010).

The purpose of the current review is to provide a synthetic view of the application of electrochemical techniques for screening pharmaceuticals. Several preliminary considerations should be made:

- Based on the IUPAC's definition of activity in biomolecular screening (Proudfoot et al. 2011), the term screening can be applied to any assay that produces a response or signal of the analyte(s) above a defined threshold at a tested concentration. In a more restricted meaning, screening could be defined as any assay devoted to producing a set of signaling features able to characterize a given analyte (or family of analytes) within a given matrix where potential interferents could be present. Even using this definition, a large number of analytical methods devoted to determine one or several pharmaceuticals in different matrices have been reported. Mainly, either voltammetric, amperometric and potentiometric sensing is used in such determinations. For reasons of similarity in electrode materials, this report will be limited to amperometric and voltammetric sensors.
- The current review is focused on voltammetric methods, those involving the record of the current response of an electrochemical cell as a function of the applied potential upon application of a certain time-dependent potential input, and amperometric methods, where only the current response of the cell upon application of a constant or a pulsed potential input is measured. In the last years, a plethora of new

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materials have been developed. These are able to be used in voltammetric/amperometric sensing and include conducting polymers (Malhotra et al. 2006), carbon nanotubes (Jacobs et al. 2010) and graphene (Shao et al. 2010, Wu et al. 2013), metal nanoparticles and quantum dots (Pumera et al. 2007, Siangproh et al. 2011), and other nanomaterials with molecular dimensions (Mousty 2004). Much of this research has been focused on several biochemical compounds: mainly glucose, ascorbic acid, dopamine, uric acid, hydrogen peroxide and NADH, several of which can be regarded as active components of pharmacological formulations. The current report, however, will be focused on pharmaceuticals other than these compounds extensively treated in the electroanalytical chemistry literature, ascribed to the following families: analgesics, anesthetics, anxiolytics, anorexics, antibiotics, antimalarials, anti-inflammatory compounds, antidepressants, antihypertensives, antivirals, antifungals, diuretics, hypoglycemics, laxatives and stimulants. It is pertinent to note that the number of publications dealing with this matter is growing increasingly.

– The electrochemical screening of pharmaceuticals by solution phase voltammetry and amperometry mainly involves stripping voltammetric and pulse amperometric methods. A recent review and textbook (Uslu and Ozkan 2011, Ozkan 2012) reports the most important voltammetric methods for the screening and determination of pharmaceuticals in different matrices of pharmacological and clinical interest. The application of the voltammetric and amperometric methods by using different techniques and working electrodes are mostly focused on the determination of the drugs as a main active principle in dosage forms. Another application field is the analysis of biological fluids for screening of the drugs as a diagnostic tool. The existing voltammetric/amperometric methods will be reviewed according to two classification criteria: the pharmaceutical classes and the types of working electrodes used for their analytical determination. The current report will be focused "purely" on electrochemical methods with no participation of biological components (enzymes, antibodies, etc.); i.e., bioelectrochemical sensing will be bypassed here.
– Electrochemical methods can be applied hyphenated with other techniques (typically, spectroelectrochemistry and electrochemical luminiscence) and coupled with separation methods as detector arrangements. This last is the case of electrochemical detection applied to high performance liquid chromatography (HPLC). Capillary electrophoresis, an important electrochemical-separation technique can also be used for screening pharmaceuticals (De Carvalho et al. 2014). All these techniques will also be omitted in this report.
– Electrochemical methods are being used increasingly for monitoring the pharmacological activity of drugs and obtaining mechanistic information on that activity. The electrochemical screening of drugs with anticancer activities was recently reviewed by Rauf et al. (2005), and the use of nucleic acid-based electrochemical sensors has been summarized by Labuda et al. (2010). For obvious reasons of space, these aspects will not be treated here.

Electrochemical methods for determining pharmaceuticals are dominated by voltammetric and amperometric sensors, accompanied by a less numerous representation of potentiometric sensors, but other electrochemical techniques such as scanning electrochemical microscopy (SECM) have been proposed for the analysis of pharmaceuticals (Lima et al. 2013). Electrochemical impedance spectroscopy, which is increasingly used for impedimetric sensing (Bonanni and del Valle, 2010) is frequently used for characterizing modified electrodes. The most extended voltammetric techniques are cyclic, differential pulse and square wave voltammetry. Among others, data treatment involves background subtraction, deconvolution and derivation techniques.

Types of electrodes

Although a part of voltammetric and amperometric sensors is constituted by unmodified electrodes (mercury, platinum, gold, glassy carbon, etc.), much of the recent research is focused on a variety of chemically modified electrodes. Following Durst et al. (1997), a chemically modified electrode is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic or polymeric film of a chemical modifier and by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film. This definition encompasses surface modified electrodes where modification consists of coating the electrode surface with a thin film of a specified material.

It is convenient to distinguish between composites, systems formed by addition of a binder to a mixture of two or more components, and functionalized materials prepared by chemical attachment of functional groups to the pristine electrode modifier (Doménech-Carbó 2010). Optionally, the base electrode can also be functionalized. Table 1 summarizes the main types of electrodes, classified according to an operational criterion, used for determining pharmacological compounds by means of voltammetric and amperometric methods. It is pertinent to note that the variety of nanocomposite formulations is enormous. Among them, multilayers of single-walled and multi-walled carbon nanotubes, graphene and/or graphene oxide, metal nanoparticles, often embedded into polymeric binders have been frequently reported. Table 1 includes a representative but non-exhaustive selection of nanocomposites. The analytical performance of carbon paste electrodes (Svancara et al. 2009), glassy carbon electrodes film-modified with acidic functionalities (Desimoni and Brunetti 2012), clays (Mousty 2004) and metal nanoparticle-modified electrodes (Oyama 2010) have been reviewed recently.

In general, the enhancement of the sensitivity in the analyte-localized signals results from the increase of the specific surface area of the electrode (effect typical of nanoparticulate and nanoporous materials) and the redox-mediating effect of the electrode modifier, the number of active sites for electrocatalysis being crucial in this regard. The attachment of the modifier to the surface of the base electrode (generally glassy carbon, Au or ITO) can be achieved via linker molecules (typically alkanethiols), self-assembled sol-gel silica networks, polymers and other methods resulting in integrated nanoarchitectures (Oyama 2010). It is pertinent to remark that the sensing properties of nanocomposite-modified electrodes depend, apart from the conducting properties of the individual modifiers, on the particle size, density and textural properties of the deposit. These properties can be studied using electrochemical impedance spectroscopy (EIS), a technique which has become popular for characterizing modified electrodes (Go and Pyun 2007).

Analytical methods

Analytical targets and strategies

Much research is devoted to the determination of individual pharmaceuticals in comercial formulations where in general, accuracy, repeatability, reproducibility and robustness are the main analytical demands. Determinations of pharmaceuticals or their metabolites (Baranowska and Koper 2009, Naggar et al. 2012) in biological fluids and other matrices (for instance, as contaminants in water) are in general more exigent in terms of sensitivity and selectivity, and attention must be paid to interference and matrix effects. The gross of the reported methods are devoted to determinations in pharmaceutical formulations, and can be eventually extended to biological fluids. Application to pharmaceutical suspensions as well, however, is rare (Carapuca et al. 2005). Several reports directly treat electrochemical control and assessment of quality (Lencastre et al. 2006, Liu et al. 2012).

Explicit application for determination of drugs in human urine (El-Maali 2000, Garcia-Fernandez et al. 2000, Fernandez Torres et al. 2002, Kasim et al. 2002, Shih et al. 2002, Hilali et al. 2003, Morais et al. 2003, Reddy and Sreedhar 2003, Razak 2004, Goyal et al. 2006, Ensafi and Hajian 2008a,b, Daneshgar et al. 2009a,b, Muralidharan et al. 2009, Tyszczuk and Korolczuk 2009a,b, Behpour et al. 2010, Ensafi et al. 2011, Zayed 2011, Gopu et al. 2012, Khaskheli et al. 2012, Gupta et al. 2013a,b, Lakshmi and Vedhi 2013, Švorc et al. 2013) and human serum (Radi et al. 2001, Sreedhar et al. 2001, Yilmaz et al. 2001, Altinoz and Nemutlu 2002, Uslu and Ozkan 2002, El-Hefnawy et al. 2003, Ghoneim et al. 2003, Coruh and Ozkan 2006, Wang et al. 2006, Altun et al. 2007, Beltagi et al. 2007a,b; El-Desoky 2009, Alarfaj 2013, Arvand and Gholizadeh 2013) are numerous. In contrast, few reports are addressed to determinations in cerebrospinal fluid (Jimenez Palacios et al. 2003), bovine muscle and serum (Ammida et al. 2004, Barbosa et al. 2006), and milk (Billova et al. 2003).

Although interference studies usually accompany analytical studies, few reports are addressed to account for specific interference processes. An example of this kind of contribution would be the determination of lovastatin in the presence of H_2O_2 in pharmaceuticals, human urine and serum (Xu and Song 2004).

Another analytical target of interest is the determination of pharmacological compounds treated as contaminants in foods (Masawat and Slater 2007, Ni et al. 2011, Mersal 2012) and herbal formulations (De Carvalho et al. 2010a,b,c, De Carvalho et al. 2013). Screening in leaves (Komorsky-Lovrić and Novak 2009) and blackberry, raspberry, strawberry, pomegranate, and sweet and blue potatoes, using solid state electrochemistry (*vide infra*) has been reported by Komorsky-Lovrić and Novak (2009, 2011).

The majority of reported approaches involve the performance of the electrochemical measurement in a solution of the analyte and/or the real samples from biological

Table 1 Summary of the main types of electrodes used for voltammetric and/or amperometric screening of pharmaceuticals.

Type of electrode	Sub-types	Examples
Unmodified electrodes	Mercury electrodes and amalgam electrodes	Nouws et al. 2007, Pecková et al. 2009
	Metallic electrodes (Pt, Au)	De Carvalho et al. 2013
	Carbon electrodes (glassy carbon, pyrolitic graphite, screen-printed graphite, etc.)	Mozo et al. 2012, Raoof et al. 2012, Merli et al. 2013
	Boron-doped diamond electrodes	Ardelean et al. 2013
	Others (ITO, FTO, etc.)	Armijo et al. 2010
Carbon paste electrodes (CPEs)	Carbon pastes	Stefan-van Staden et al. 2010, Liu et al. 2012
	Modified carbon pastes	Heli et al. 2012, Arvand and Fallahi 2013
	Carbon pastes with advanced binders	Gupta et al. 2013a,b
Electrochemically pretreated electrodes	Grafting/functionalizing carbon electrodes	Li et al. 2012, Yang et al. 2012a,b
	Metal-plated electrodes	Tyszczuk 2009, Pournaghi-Azar et al. 2010
	Anodization of metal electrodes	Wolfart et al. 2013
Coatings on solid electrodes	Self-assembled monolayers (SAMs)	Temsamani et al. 1997
	Host-guest monolayers	Saraswathyamma et al. 2008
	Polymer films	Kalimuthu and John 2010
	Lipid films	Nikolelis and Mitrokotsa 2004
Particulate deposits on solid electrodes	Microparticulate deposits	Yin et al. 2012
	Metal nanoparticles	Nair et al. 2009, Atta et al. 2011a,b
	Semiconductor (typically metal oxide) nanoparticles	Zidan et al. 2011, Ye et al. 2012
Deposits of nanostructured carbon-based materials:	Fullerenes	Goyal and Singh 2006
	Carbon nanotubes	Mohammadi et al. 2013
	Graphene (graphene oxide, reduced graphene oxide)	Kang et al. 2010, Li et al. 2012
Deposits of nanocomposite layers and multilayers combining the above materials	Polymer binding of carbon-based materials	Yin et al. 2010, Babael et al. 2011, Filik et al. 2013
	Metal nanoparticles/carbon-based materials	Tsierkezos et al. 2014, Li et al. 2014

fluids, pharmaceutical formulations etc., using a suitable electrode and external calibration or standard additional strategies. Other analytical strategies have been reported in recent literature. These are summarized in Table 2.

In this context, screening of different compounds and/or families of compounds can be obtained from discriminating voltammetric responses. In several cases, separated voltammetric signals can be obtained for the different compounds, as is the case of screening of anxiolytics as adulterants in herbal formulations using mercury electrodes (De Carvalho et al. 2010c). Figure 1 shows the voltammetric signals of a 1,4-benzodiazepine mixture and amfepramone in Ringer buffer at pH 10.0. Modification of the electrolyte composition, selective enhancement/depletion of compound-characteristic signals by using electrodes modified with modifiers exhibiting high selectivity, combination of voltammetric data with separation techniques such as liquid chromatography (Ağin et al. 2013) or solid state voltammetry (*vide infra*) can be used. Discrimination between different analytes is, in principle, easier using voltametry measurements whereas amperometric measurements provide higher sensitivity.

Voltammetric methods

The group of antibiotics has been the most extensively studied for voltammetric screening. Determinations at mercury electrodes have been reported for cefadroxil (Al-Ghamdi et al. 2009), cefatemet (Kapetanovic et al. 2000a,b, Aleksic et al. 2003), cefazolin (El-Desoky et al. 2005), cefdinir (Jain et al. 2007a,b, 2008), cefepine (Palacios et al. 2000, Jimenez Palacios et al. 2003, Kumar et al. 2005), cefixime (Jain et al. 2010), cefminox (Hilali

Table 2 Several electrochemical strategies complementing the direct voltammetric/amperometric measurements at macroelectrodes in analyte solutions reported in recent literature for determining pharmaceuticals.

Strategie	Example of application
Based on electrolyte modifications	
Use of micellar media	Quantification of menadione in sodium dodecyl sulfate micellar media (Ziyatdinova et al. 2013)
Addition of catalysts	Determination of metformine in pharmaceuticals catalyzed by Cu(II) (Tian and Song 2007)
Based on electrode modifications/arrangements	
Use of micro and nanoelectrodes	Penicillin G determination at gold ultramicroelectrode (Norouzi et al. 2006a,b)
Advanced electrode pretreatments and/or electrode functionalizations	Determination of paracetamol, caffeine and orphenadrine at a cathodically pretreated boron-doped diamond Electrode (Pires Eisele et al. 2013)
Host-guest recognition strategies of electrode modification	
Molecularly imprinted polymers	Determination of doxycycline at molecularly imprinted overoxidized polypyrrole electrodes (Gürler et al. 2013)
Cyclodextrins	Determination of levodopa and carbidopa at cyclodextrin-modified electrodes (Abbaspour et al. 2011)
Screen printed electrodes	Paracetamol determination (Fanjul-Bolado et al. 2009)
Based on modifications of the measurement technique	
Hydrodynamic voltammetry	Selection of Pharmaceutical Antioxidants by Hydrodynamic Voltammetry (Webster et al. 2012)
Constant current coulometry	Determination of antioxidants (Ziyatdinova et al. 2012)
Flow-injection with multiple-pulsed amperometric detection	Verapamil determination (Ortuño et al. 2005)
Nanochannel-based arrangements	Nagaraj et al. 2014

et al. 2003), cefonicid (Radi et al. 2003), cefoperazone (Billova et al. 2003), cefpodoxime (Aleksic et al. 2004), cephalosporins (Prasad and Gupta 2000, Abo El-Maali et al. 2005), cephalotin (Al-Ghamdi et al. 2004), cholarphenicol (Dumitrescu et al. 2001), and clarithromycin

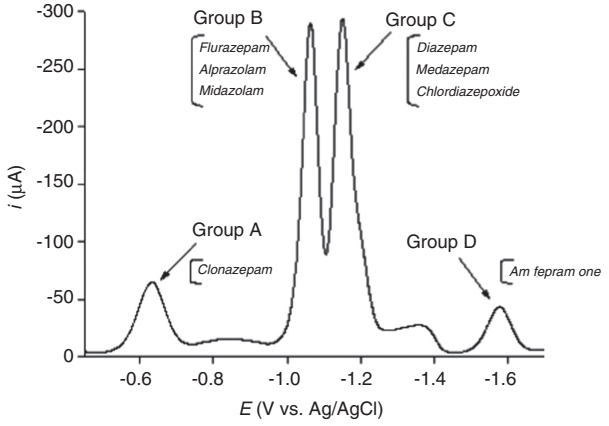

Figure 1 Voltammetric signals at HMDE of a 1,4-benzodiazepine mixture and amfepramone in Ringer buffer at pH 10.0. clonazepam=6×10^{-5} M; amfepramone=3×10^{-5} M; other benzodiazepines: 1×10^{-5} M. Negative-going potential scan; pulse mplitude 50 mV, pulse duration 40 ms, scan rate of 50 mV s^{-1}. From De Carvalho et al. 2010c, with permission.

(Ghoneim and El-Attar 2008), imipenem (Fernandez-Torres et al. 2008), isoniazid (Ghoneim et al. 2003), josamycin (Belal et al. 2002a), shiomarin (Gu, 2002), mitomycin (Sreedhar et al. 2001), nalidixic acid (Ibrahim et al. 2002), nitrofurantoin (Hammam 2002, Jain et al. 2009a,b), nitroxaline (Ghoneim et al. 2011), rifamycin (Alonso et al. 2000), rifampicin (Alonso Lomillo et al. 2002), sulfonamides (Sabry 2007), tobramycin (Sun et al. 2005), trimethoprim (Carapuca et al. 2005) and vancomycin (Belal et al. 2001a,b). Determination of floxacines at mercury electrodes was reported by several authors, (Kapetanovic et al. 2000a,b, Rizk et al. 2000, Vilchez et al. 2001, 2003, Navalón et al. 2002, Beltagi 2003, Solangi et al. 2005, Abdel Ghani et al. 2007, Al-Ghamdi 2009, El-Desoky 2009). Simultaneous determination of several tetracycline antibiotics was described by Ni et al. (2011). Determinations at glassy carbon electrodes have been proposed for azithromycin (Nigovic and Simunic 2003, Nigovic 2004), cefadroxil (Ozkan et al. 2000), cefixime (Golcu et al. 2005), cefoperazone (Dogan et al. 2009a), cefotaxime (Dogan et al. 2009b), dapsone (Manisankar et al. 2001), erythromycin (Wang et al. 2000), levofloxacin (Radi and El-Sherif 2002), nifuroxazide (Toral et al. 2004), and floxacines (Ghoneim et al. 2001, Kumar et al. 2006). Carbon paste electrodes were also used for azithromycin (Farghaly and

Mohamed 2004), ceftazidime (El-Maali 2000), doxycycline (Attia and Saber 2011), floxacines (Ries et al. 2005) and others (Gutierrez-Fernandez et al. 2004, Hammam et al. 2004, Wahdan 2005, Souza et al. 2012). Determinations at boron-doped diamond electrodes were reported by Uslu et al. (2008), Andrade et al. (2009), De Lima-Neto et al. (2010), and Švorc et al. (2012a,b). Gold electrodes and ultramicroelectrodes were used by few authors (Palaharm et al. 2003, Norouzi et al. 2006a,b, 2008).

The use of nanocomposites has also been considerably developed for the determination of anti-inflammatory drugs. Carbon nanotubes were the favorite electrode modifiers on edge plane pyrolitic graphite (Goyal and Bishnoi 2009, Goyal and Bishnoi 2010a,b, Goyal et al. 2010a,b,c,d,e) and carbon paste (Abbaspour and Mirzajani 2007) Wang et al. (2006). Composites of carbon nanotubes with Schiff base metal complexes (Amiri et al. 2011), $Cu(OH)_2$ (Arvand et al. 2012) and cysteic acid (Wang et al. 2006) have been also reported. Electrode modification with polypyrrole film (Santhosh et al. 2007), montmorillonite (Beltagi 2009), Ni-curcumin (Heli et al. 2009), iron complexes (Hasanzadeh et al. 2012), β-cyclodextrin (Balaji et al. 2008) and chitosan-doped carbon nanoparticles (Shahrokhian et al. 2010) complete the scope of anti-inflammatory screening.

Electrode modification strategies involved with conducting polymers combined with micellar media (Brahman et al. 2013) to carbon paste electrodes modified with complexes (Bergamini et al. 2006), ferrocenedicarboxylic acid alone (Khalilzadeh et al. 2009) or combined with carbon nanotubes (Fouladgar et al. 2011) or carbon nanotubes alone (Shahrokhian and Amiri 2007, Shahrokhian and Asadian 2010, Arvand et al. 2011). Glassy carbon electrodes were also used as substrates for electrode modification with carbon nanotubes (Fotouhi and Alahyari 2010, Jain et al. 2011a,b), polymers (Msagati and Ngila 2002, Huang et al. 2008, Yang et al. 2008). The deposition of carbon nanotubes on gold was less usual (Yan et al. 2011).

Modifiers involved with β-cyclodextrin (Reddy and Sreedhar 2003) and double stranded DNA (dsDNA) (Radi et al. 2005) on dysprosium hydroxide nanowires (Daneshgar et al. 2009a,b). Carbon nanotubes decorated with spinels and related inorganic solids (Ensafi et al. 2012a,b, 2013a,b). Screen printed electrodes (Ammida et al. 2004, Bergamini et al. 2010) and molecularly imprinted polymers (Gürler et al. 2013) were also reported. Graphene plus ionic liquid composite (Peng et al. 2011) and lead films on glassy carbon electrode (Korolczuk and Tyszczuk 2007, Tyszczuk and Korolczuk 2009a,b) were other types of electrode modifications used for screening of antibiotics.

Voltammetric methods for the screening of chemicals in pharmaceutical formulations and biological fluids reported since 2000, organized by families of drugs other than antibiotics, are summarized in Table 3. In the case of analgesics, acetaminophen is by far the most studied drug, alone and simultaneously, with other drugs. Apart from unmodified carbon electrodes, glassy carbon and boron-doped diamond electrodes, a variety of nanocomposites have been reported for this purpose (Goyal et al. 2005, Ghorbani-Bidkorbeh et al. 2010, Fan et al. 2011, Samadi-Maybodi et al. 2011, Khaskheli et al. 2012). Methods for electrochemical determination of morphine have also been proposed, including metal complexes (Teixeira et al. 2004; Teixeira and Dadamos 2009) and minerals (Shih et al. 2002, Gualandi et al. 2011), among others (Ensafi et al. 2013a,b).

Anestethics, anorexics and anxiolytics have been also studied. The analysis of anxiolytics illustrates the importance of developing screening methods because most anxiolytics are 1,4-benzodiazepines (alprazolam, clonazepam, diazepam, flurazepam, etc.) having a common active unit. Because of their chemical and structural similarity, but not equivalent pharmacological activity, there is need for developing methods for individualizing such compounds in both pharmaceutical formulations and biological fluids. In this regard it would be pertinent to emphasize the significance of mechanistic studies on the voltammetric response (Garrido et al. 2008, Pournaghi-Azar et al. 2010, Altunöz-Erdoğan 2013).

In the case of antidepressants composites based on carbon nanotubes using β-cyclodextrin (Ferancova et al. 2000), graphite-polyurethane (De Toledo et al. 2006) and polymer films (Lakshmi and Vedhi 2013) have been described, but a biosensor based on silica modified with niobium oxide, aluminium and DNA embedded into carbon paste has been recently described (Marco et al. 2013), and fullerenes (Goyal and Singh 2006), mordenite-type zeolite (Arvand et al. 2010), clays (Madhusudana Reddy and Jayarama Reddy 2004), ferrocenedicarboxylic acid (Karimi-Maleh et al. 2010), metal complexes (Shahrokhian et al. 2005, Eleotério et al. 2012), metal nanoparticles (Goyal et al. 2006, Behpour et al. 2010, Stoiljković et al. 2012) have been reported with the determination of antihypertensive pharmaceuticals.

Voltammetric methods for the determination of antiviral, antifungal and diuretic ragents are also listed in Table 3. In this last group, graphite-polyurethane composite (Seman et al. 2008) and ferrocenedicarboxylic acid modified carbon paste electrodes (Karimi-Maleh et al. 2009) have also been used.

Table 3 Voltammetric methods for the screening of important families of chemicals in pharmaceutical formulations and biological fluids reported since 2000.

Family (representative analytes)	Electrode	Reference
Analgesics	C	Garcia-Fernandez et al. 2000, Ni et al. 2004, Uliana et al. 2010, Ozcan and Sahin 2011
(acetaminophen, morfine)	GC	Garrido et al. 2002, Garrido et al. 2003a,b, Torriero et al. 2006, Kashefi-Kheyrabadi and Mehrgardi 2012
	BDD	Wangfuengkanagul and Chailapakul 2002, Lourenção et al. 2009, Sartori et al. 2009, Švorc et al. 2012a,b, 2013
	CNT	Wan et al. 2009a,b, Goyal et al. 2010a,b,c,d,e, Sanghavi and Srivastava 2010, 2011a,b, Shahrokhian and Asadian 2010, Habibi et al. 2011, Lu and Tsai 2011, Arvand and Gholizadeh, 2013 Ghadimi et al. 2013
	Hg	Niazi and Yazdanipour 2008
	Au NP	Atta et al. 2011a,b
	P	Atta and El-Kady 2009, Muralidharan et al. 2009, Gopu et al. 2012, Thomas et al. 2013
	Other	Goyal et al. 2005, Ghorbani-Bidkorbeh et al. 2010; Fan et al. 2011, Samadi-Maybodi et al. 2011, Khaskheli et al. 2012
Anestethics (lidocaine,	BDD	Oliveira et al. 2007
procaine)	Hg	Ali et al. 2000, mercury
	GCE/P	Li et al. 2003
Anxiolytics	CPE	Lozeno-Chaves et al. (2006)
	Hg	Dos Santos et al. 2002, El-Hefnawy et al. 2004a,b, Sabry et al. 2004, Hammam 2007, Ghoneim et al. 2008, Jain et al. 2009a,b, Naggar et al. 2012
	Pb film	Tyszczuk 2010
Anorexics	Hg	De Carvalho et al. 2007, 2010a,b,c
Antimalarials	CP	Radi 2005, Mashhadizadeh and Akbarian 2009
	GC	Arguelho et al. 2003, 2005, Uslu et al. 2005a,b,c, Jain and Vikas 2011a,b
Anti-inflammatory	CP	Malode and Nandibewoor 2013a,b
	GC	Yilmaz et al. 2001, Altun et al. 2007, Farhadi and Karimpour 2007, El-Sayed et al. 2009, Ghavami and Navaee 2012
	BDD	Suryanaron et al. 2005
	Hg	Radi et al. 2001, Sayin and Kir 2001, Altinoz and Nemutlu 2002, Beltagi et al. 2002, 2007a,b; Kasim 2002; El-Enamy et al. 2003, El-Hefnawy et al. 2003, Ghoneim and Beltagi 2003, Ghoneim and Tawfik 2003a,b, Hammam 2007, Zayed 2011
	Au	Norouzi et al. 2007, Malode and Nandibewoor 2013a,b
	Pt	Uslu et al. 2001, Adhoum et al. 2003
Antidepressants	GC	Uslu and Ozkan 2002, Lencastre et al. 2006, Turhan and Uslu 2008, Erdogan et al. 2011, Altunöz-Erdoğan et al. 2013
	CNT	Hegde et al. 2009, Sanghavi and Srivastava 2011a,b
	Hg	Vela et al. 2001, El-Enany et al. 2002, Ozaltin et al. 2002, Ozaltin et al. 2002, Morais et al. 2003, Nouws et al. 2005a,b, 2006a,b, 2007, Jain and Radhapyari 2008, Erdogan et al. 2011, Altunöz-Erdoğan et al. 2013
	Au^m	Norouzi et al. 2006a,b
Antihypertensive	CP	Arranz et al. 2000a,b, Radi 2001, Radi et al. 2004a,b, Kazemipour et al. 2009, Patil et al. 2009, Attia, 2010
	GC	Ozkan 2002, Dogan et al. 2004, Gazy 2004, Dogan and Ozkan 2005, Kumar et al. 2005, Radi and Elmogy 2005, Öztürk et al. 2011, Ensafi and Arabzadeh 2012
	CNT	Goyal et al. 2008, Goyal and Bishnoi 2010a,b, Ensafi et al. 2011, 2012a,b, Jara-Ulloa et al. 2012, Karimi-Maleh et al. 2012, Stoiljković et al. 2012
	BDD	Sartori et al. 2010
	Hg	Al-Majed et al. 2000, Belal et al. 2001a,b, 2002a, 2001b, 2003, Dumitrescu et al. 2001, Ghandour et al. 2001, 2002, El-Ries et al. 2002, Kasim et al. 2002, Tamer et al. 2002, Demircigil et al. 2003, Ghoneim and Tawfik 2003a,b, Ioannides et al. 2003, Prieto et al. 2003, Razak et al. 2003, Parham and Zargar 2005, Ensafi and Hajian 2008a,b, Suslu et al. 2008, 2009, El-Desoky et al. 2011, Gupta et al. 2011, Alarfaj 2013
	Pt	Altiokka 2001, Ziyatdinova et al. 2006
Antiviral	GC	Uslu and Ozkan 2004, Dogan et al. 2006, Uslu et al. 2005a,b,c, 2006
	Hg	Vacek et al. 2004, Dogan et al. 2005, 2008, Jain et al. 2007a,b, Skrzypek et al. 2009, Leandro et al. 2010, Gaber et al. 2013

(**Table 3** Continued)

Family (representative analytes)	Electrode	Reference
Antifungal	CP	Aki et al. 2005
	GC	Shamsipur and Farhadi 2000, 2001
	CNT	Radi 2001
	Hg	Arranz et al. 2003, Ibrahim and El-Enany 2003, El-Desoky 2005
	Ag(Hg)	Dantas et al. 2010
	Au, Pt	Shamsipur and Farhadi 2000, 2001
Diuretics	CP	Radi 2001
	GC	Razak 2004
	CNT	Malode et al. 2012
	Hg	El-Hefnawy et al. 2004a,b, Hammam 2004, Al-Ghamdi et al. 2008, Ensafi and Hajian 2008a,b
Hypocholesterolemic (estatins)	CP	Abbar and Nandibewoor 2013
	GC	Ozkan and Uslu 2002, Coruh and Ozkan 2006, Dogan-Topal et al. 2007, 2009a,b
	BDD	Dogan-Topal et al. 2007
	Hg	Fernandez Torres et al. 2002, Xu and Song 2004, Yardimci and Ozaltin 2004, Nigovic 2006, Korany et al. 2008, Neves et al. 2008, Nigovic et al. 2008, Nigovic and Pavkovi 2009, Eskiköy et al. 2011
Hypoglycemics	GC	Radi et al. 2004a,b, El-Ries et al. 2008, Badawy et al. 2010
	Hg	Ghoneim et al. 2007
Laxatives (bisacodyl) and stimulants (caffeine)	CP	Mersal 2012
	GC	Ozkan et al. 2004, Uslu et al. 2005a,b,c
	CNT	Sanghavi and Srivastava 2010, Liu et al. 2013
	Hg	Berzas et al. 2000, Rodriguez et al. 2004
	Au^m	Daneshgar et al. 2009a,b
	Pb film	Tyszczuk and Korolczuk 2010

C, unmodified carbon; CP, carbon paste; GC, glassy carbon; BDD, boron-doped diamond; CNT, carbon nanotubes; P, polymer electrodes. The superscript ^{m} denotes microelectrodes.

Several general aspects dealing with the voltammetric screening of pharmaceuticals can be applied:

- Electrode modification strategies generally used to promote an electrocatalytic effect on the oxidation or reduction of the analyte. In most cases, synergistic effects appear between different electrode modifiers. This permits enhancement of the sensitivity (and often the selectivity) of the determinations relative to unmodified electrodes, and electrodes modified with each one of the modifiers separately. This is the reason for testing a wide variety of combinations of modifiers (metal nanoparticles, polymers, carbon nanotubes, graphene, etc.) for working electrode preparation.
- Apart from sensitivity and selectivity, the repeatability and reproducibility of the sensing method and the robustness of the electrode are important aspects to be considered in order to evaluate the analytical performance of the electrochemical method. It is pertinent to note that the environmental protection ("green" sensors), economy and ease of preparation ("one-pot" synthetic procedures) are important factors acting as guidelines for electrode modification (Li et al. 2014).
- However, much less attention has been devoted to the detailed description of the electrocatalytic pathways involved in voltammetric/amperometric sensing. This obeys, in part, with the essentially operational nature of much of the research involved, focused on the analytical aspects with no need for a detailed knowledge of the involved electrochemical pathway. However, it appears reasonable to put emphasis on the interest of better understanding of the nature of the involved electrochemical processes, in particular for explaining the selectivity effects and the synergistic interactions among different electrode modifiers.
- Screening of different pharmaceuticals is, in general, obtained from voltammetric signals appearing at sufficiently separated potentials permitting the detection, and even quantification, of the different components. A typical example is the discrimination between paracetamol and its glucuronide and

sulfate metabolites (Baranowska and Koper 2009). Figure 2 shows an example of the voltammetric discrimination of 1,4-benzodizepines in the presence of amfepramone using differential pulse voltammetric measurements at the hanging mercury drop electrode (De Carvalho et al. 2010c).

Apart from electrode modification, variations of the pH of the media, selection of the electrochemical mode (linear scan vs. pulsed techniques, etc.) and optimization of the electrochemical parameters (scan rate, frequency, etc.) can significantly alter the discriminating ability of a given electrochemical sensor.

Amperometric methods

Amperometric methods for the screening of pharmaceuticals appear frequently associated with flow injection analysis and high performance liquid chromatography. In the case of analgesics: boron-doped diamond electrodes were used for determinations of acetaminophen (Wangfuengkanagul and Chailapakul 2002, Silva et al. 2011), acetylsalicylic acid (Miranda et al. 2012) and codeine (Gimenes et al. 2013). Other determinations involve glassy carbon (Garrido et al. 2000, 2003a,b, Dos Santos et al. 2009, Au (Dos Santos et al. 2008), Cu (Quintino et al. 2002a,b) and unmodified carbon paste electrodes (Marcolino-Júnior et al. 2003). Different types of thiol-modified Au (gold) electrodes (Pedrosa et al. 2006, Nair et al. 2009) were used, among other electrode modifications (Catarino et al. 2002, Cervini and Cavalheiro 2008a,b, Razmi and Harasi 2008, Fanjul-Bolado et al. 2009, Gualandi et al. 2011).

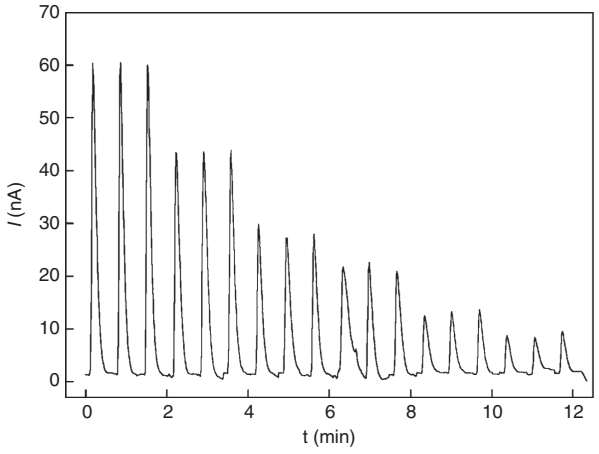

Figure 2 Amperometric response of carbon paste electrode in flux injection analysis of benzocaine with concentration 10; 8; 6; 4; 2; 1×10^{-5} mol l^{-1}. Carrier solution Briton-Robinson buffer pH 4 containing 80% of methanol (v/v), flow rate 1.0 ml min^{-1}, Detection potential +1.2 V, injected 20 µl of benzocaine solution. From Dejmkova et al. 2011, with permission; copyright © 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Reported amperometric methods for determining anestethics include screen-printed carbon electrodes (Bergamini et al. 2007), carbon paste (Dejmkova et al. 2011) and multi-walled carbon nanotubes on glassy carbon (Guo et al. 2013). In the case of anxiolytics, determination of buspyrone hydrochloride at multi-walled carbon nanotubes-modified electrode was reported (Cheemalapati et al. 2013).

Antibiotic screening methods are almost unanimously focused on determinations in pharmaceutical formulations. Glassy carbon (Palomeque and Ortíz 2007, Pfaffen et al. 2013), paraffinated graphite (Perantoni et al. 2011), carbon paste (Dejmkova et al. 2013), boron-doped diamond (Wangfuengkanagul et al. 2004) and, particularly, gold (Palaharm et al. 2003, Charoenraks et al. 2004, Codognoto et al. 2010) and screen-printed gold (Masawat and Slater 2007) were reported electrode materials. Electrode modification with metalloporphyrin (Gong et al. 2003), cyclodextrin (Lomillo et al. 2005), carbon nanotubes (Chen et al. 2012), among others (Won et al. 2013), have been reported. Electrode modification strategies are becoming increasingly complicated, thus Lian et al. (2012) have reported a molecularly imprinted polymer film at chitosan-platinum nanoparticles/graphene-gold nanoparticles double nanocomposites for sensing erythromycin in real samples.

Also, amperometric methods have been applied to the group of antidepressants, represented by the determination of citalopram on glassy carbon (Nouws et al. 2008), and to antihypertensive pharmaceuticals: Atenolol was determined in pharmaceutical formulations using graphite-polyurethane composite (Cervini et al. 2007) and captopril was determined with carbon paste (Marcolino et al. 2009). Determination in urine was reported by Ensafi and Arabzadeh (2012) using glassy carbon electrodes.

Determinations of anti-inflammatory drugs involved boron-doped diamond (Gimenes et al. 2011, 2013), tubular bismuth film (Rodríguez et al. 2007) and glassy carbon (Silva et al. 2007, Stefano et al. 2012), and the amperometric screening of amiloride, chlorthalidone, furosemide and hydrochlorothiazide diuretics in herbal formulations on gold electrodes was reported recently by De Carvalho et al. (2013). Hypocholesterolemic products were determined in pharmaceuticals formulations using unmodified (Neves et al. 2008) and multi-walled carbon nanotubes-modified glassy carbon electrodes (Jalali et al. 2010). Figure 2 shows a typical amperometric graph illustrating the current response of carbon paste electrode in flux injection analysis of benzocaine with injections of the

analyte in decreasing concentrations taken from Dejmkova et al. (2013).

Finally, determination of stimulants at boron-doped diamond electrodes (Silva et al. 2011, Júnior et al. 2012) and nafion-modified glassy carbon electrodes(Torres et al. 2014) were proposed. Kan et al. (2012) described a caffeine amperometric sensor consisting of molecularly imprinted polymers associated with carbon nanotubes and gold nanoparticles.

Amperometric sensing involves the application of potential constant steps under conditions of diffusion control of the electrochemical experiment. Stability and repeatability of the measurements are obvious analytical demands often requiring electrode cleaning/regeneration, an aspect that is overlayed in most literature. The linearity of the response is another crucial aspect in amperometric (and voltammetric) measurements. In this regard, it is pertinent to indicate that in most cases, two different linear regimes at relatively low, and relatively high, analyte concentrations are reported. The appearance of two almost linear regimes can be attributed, in principle, to the occurrence of different diffusion regimes, but this would be another aspect requiring more detailed research.

Solid state techniques

Much pharmaceutical products are prepared and commercialized as solid materials usually constituted by a mixture of pharmacologically active components or by a mixture of the active component and a suitable excipient. Analysis of solid materials as well can reduce the time analysis and reduce opportunity for sample alteration or contamination associated with pretreatments involving digestion, dissolution, etc. of the sample. Application of electrochemical techniques for analyzing solid materials was initiated by the introduction of carbon paste electrodes by Adams (1958), and Kuwana et al. (1965). The electrochemical methods for analyzing solids have been notably expanded with the development of the voltammetry of immobilized particles (VIMP) by Scholz et al. (1989a,b), Scholz and Meyer (1998) and Scholz et al. (2005). As recently reviewed by De Carvalho et al. (2010a), this technique provides voltammetric records characterizing the composition of solid materials attached to inert electrodes (typically, paraffin-impregnated graphite electrodes) in contact with suitable electrolytes. The key point is that the solid analytes experience solid-state electrochemical processes where ion insertion/release was coupled to electron transfer so that voltammetric signals were characteristic of the chemical/mineralogical composition of the solid material, thus providing qualitative and quantitative information on the composition of mixtures of solids, mixed phases and oxidation state (Scholz et al. 2005) prompting the use of the voltammetry of microparticles as a method for electrochemical analysis of solids (Doménech-Carbó et al. 2013a).

After the application of the VIMP for screening pesticides (Reddy et al. 1996) and determining cocaine (Komorsky-Lovrić et al. 1999), this technique has been applied by Komorsky-Lovrić et al. (2003) for characterizing local anesthetics and antithusics and other pharmaceutical products (Komorsky-Lovrić and Nigović 2004, 2006, Komorsky-Lovrić et al. 1999). More recently, VIMP has been applied to determine antioxidative properties of tea leaves (Komorsky-Lovrić and Novak 2009, 2011). The VIMP methodology has been used for screening of pharmacologic adulterant classes in herbal formulations (Doménech-Carbó et al. 2013b,c) and antimalarial drugs acting via the hemozoin mechanism (Doménech-Carbó et al. 2013d).

Figure 3 illustrates the application of the VIMP for assessing the presence of adulterants in phytotherapeutic formulations (Doménech-Carbó et al. 2013b). Here, square wave voltammograms for paraffin-impregnated graphite electrodes modified with microsamples of different commercial herbal formulations were compared with the voltammograms for standards of: a) chlorpropamide, glibenclamide, and glimepiride hypoglycemics; b) furosemide (diuretic); c) lorazepam (anxiolytic) and d) femproporex (anorexic), in contact with aqueous acetate buffers. As can be seen in this figure, the different pharmaceuticals provide characteristic voltammetric profiles which permit the identification of such compounds as adulterants in commercial herbal formulations. The relevant point to emphasize is that micro- or, if necessary, nanosamples of the solid material can be transferred by abrasion to the surface of the base graphite electrode where the particles of the different materials display independent voltammetric responses. This highly sensitive methodology avoids interferences often appearing in dissolved samples and contamination and/or alteration associated with sample pretreatment, also allowing for multicomponent analysis.

VIMP can also be applied for quantitations. Relative quantitation can be derived from peak current (or peak area) ratios, whereas absolute quantitation can be achieved via standard additions experiments. The application of this technique for determining psychoactive 1,4-enzodiazepine and antidepressants drugs as adulterants in phytotherapeutic formulations has been reported recently (Doménech-Carbó et al. 2013b).

Based on the VIMP methodology, electrode modification with microparticulate deposits of solids can be used for screening analytes in solution on the basis of the

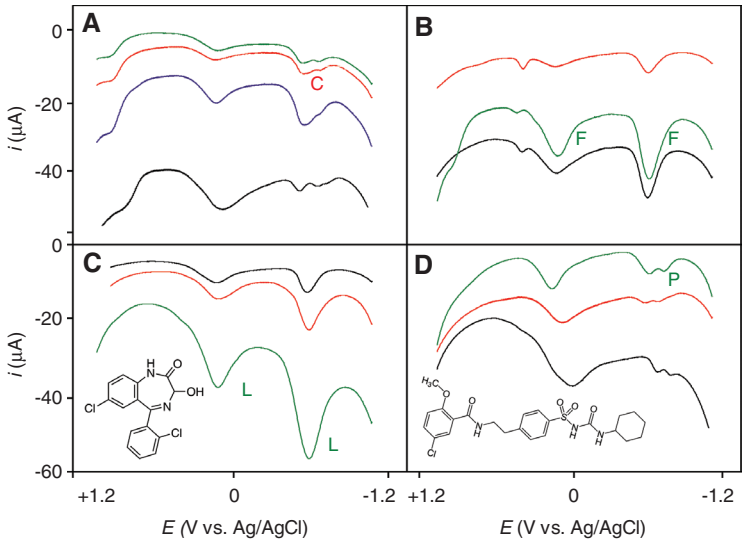

Figure 3 Square wave voltammograms initiated at +1.05 V in the negative direction for paraffin-impregnated graphite electrodes modified with different samples of herbal formulations (black lines) and standards of adulterants. A) chlorpropamide (C peaks, red), glibenclamide (green) and glimepiride (blue); B) a second sample (red) and furosemide (F peaks, green); C) a second sample (red) and lorazepam (L peaks, green); D) a second sample (red) and femproporex (P peaks, green). Electrolyte 0.25 M HAc/NaAc. Insets: structures of C) lorazepam and D) glibenclamide. Adapted from Doménech-Carbó et al. 2013b, with permission from Elsevier.

modification of the voltammetric signals of the solid modifier. This have will been the case with cysteine determination at copper hexacyanoferrate modified electrodes (Ravi Shankaran and Sriman Narayanan 1998). This methodology has been recently applied in discriminating between dsDNA, ssDNA, G-quadruplex DNA, and nucleosomal DNA (Doménech-Carbó et al. 2014).

Concluding remarks

Screening of pharmaceuticals can be considered as an important analytical target to be achieved by electrochemical methods. The number of voltammetric and amperometric methods for screening compounds of pharmacological interest has experienced an explosive growth in the last years due to the development of a plethora of modified electrodes, in particular involving a wide variety of combinations of nanostructured materials, and analytical strategies. It would be interesting to combine applied research with fundamental research in order to better understand the electrochemical mechanisms involved in electrochemical recognition processes. Apart from conventional solution electrochemistry, solid state voltammetric techniques could also be applied.

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