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Sensors and biosensors based on clay-modified electrodes — new trends

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Abstract

Clay-modified electrodes (CLMEs) have received attention in the development of electrochemical sensors and biosensors. This article reviews the use of CLME for these electroanalytical purposes. It includes an introduction to the structure of cationic and anionic clays, an overview of electron transfer occurring at CLME, and of the different modes of preparation of CLME. The analytical applications reported for CLME range from the preconcentration method applied to the detection of cationic species (i.e., metal cations) or organic molecules (i.e., water pollutants and drugs), to electrocatalytic sensors involving intercalated redox mediators in the electrochemical detection process, and finally to amperometric and potentiometric biosensors. Several enzymes have been immobilized within clay matrices and amperometric biosensors based on CLME are presented following the three modes of detection referred to as first, second, and third generation of biosensors, depending on the nature of the enzymes.

Keywords: Clay-modified electrodes; Electroanalysis; Electrochemical biosensors; Layered double hydroxides; Redox mediator; Enzyme

Abbreviations: AA, ascorbic acid; ABTS, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate; AlDH, alcohol dehydrogenase; BSA, bovine serum albumin; Cc, cobaltocenium; CE, cholesterol esterase; CEC, cationic exchange capacity; CO, cholesteroloxidase; CPE, carbon paste electrode; CV, cyclic voltammetry; DDAB, didodecyldimethylammonium; DHB, 3,4-dihydroxybenzaldehyde; DPV, differential pulse voltammetry; EQCM, electrochemical quartz crystal microbalance; FAD, flavin-adenine dinucleotide; Fc, ferrocene; Fre, flavin reductase; GA, glutaraldehyde; GOD, glucose oxidase; GCE, glassy carbon electrode; HRP, horseradish peroxidase; ITO, indium tin oxide; LaDH, lactate dehydrogenase; LDHs, layered double hydoxides; MG, methylene green; MP, maltose phosphorilase; MR, mutarotase; MV^{2+}, methylviologen; NADH, nicotinamide adenine dinucleotide; PGE, single use pencil graphite electrode; PISE, potentiometric Ion Sensitive Electrode; PMMA, polymethyl methacrylate; PPD, poly(o-phenylenediamine); PPO, polyphenol oxidase; Pr, protein; PS, organosilasesquioxane pillared laponite; Py, lead ruthenate pyrochlore ($Pb_2Ru_{2-x}Pb_xO_{7-y}$); SPE, screen-printed electrode; SWV, square wave voltammetry; TCA, trichloroacetic acid; TTF, tetrathiofulvalene; XO, xanthine oxidase.

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1. Introduction

A "chemical sensor is a small device that, as the result of a chemical interaction or process between analyte and the sensor device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal" (Stetter et al., 2003). All chemical sensors contain two basic components: a chemical recognition system (receptor) and a transducer. Biosensors are chemical sensors in which the recognition system uses a biological mechanism instead of a chemical process. A transducer transforms the response measured at the receptor into a detectable signal. Among all the chemical sensors reported in the literature, electrochemical sensors are the most attractive because of their remarkable sensitivity, experimental simplicity, and low cost. The signal from the transducer can be a current (amperometry), a voltage (potentiometry), or impedance/conductance changes (conductimetry).

Chemical layers can be used for importing a high degree of selectivity to electrochemical transducers. Chemically modified electrodes (CMEs) provide one approach to the development of these analytical devices. CMEs are important constituents of both immobilized reagent systems and sensitive layers. In trace analysis, during the accumulation reaction, CMEs preconcentrate the analyte into a small volume on the electrode, allowing lower concentrations to be measured than possible in the absence of a preconcentrated step (adsorptive stripping voltammetry). CME can also be applied to electroanalysis because of their own electrocatalytic properties and/or their capacities to immobilize electrocatalytic or biocatalytic (enzyme) reagents that improve the sensitivity and selectivity of the detection step.

The majority of modified electrodes can be obtained by chemisorption, covalent bonding, and film deposition (Murray, 1992). Among the wide range of electrode modifiers, inorganic materials, such as zeolites, silica-based hybrid materials, and clays, have attracted the attention of electrochemists, in particular for their analytical applications (Walcarius, 1996, 1998, 2001; Navrátilová and Kula, 2003).

Clays can be divided into two main classes: cationic clays that have negatively charged alumino silicate layers; and anionic clays, with positively charged hydroxide layers. The neutrality of these materials is ensured by ions, cations, or anions, depending on the clay type, in the interlayer space that balances the charge. Cationic clays are among the most common minerals on the earth's surface. They have been used for centuries to produce ceramics. Furthermore, they find applications in pharmacy, cosmetics, catalysts, adsorbents, and ion exchangers (Vaccari, 1998, 1999). These last applications are particularly useful for the development of electrochemical sensors. Most clays used at clay-modified electrodes (CLME) are Smectite clays. Their names and formula are given in Table 1 (Newman and Bown, 1987; Van Olphena and Fripiat, 1979). They can serve as matrices for electroactive ions because they are usually able to incorporate ions by an ion-exchange process, like polymeric ionomers. Moreover, adsorption of proteins on clay mineral surfaces plays a very important role not only in fields related to agricultural and environmental sciences, but also in the development of biosensors (Gianfreda et al., 2002). Layered

Table 1
Composition of clay types used at clay-modified electrodes

Clay type	Name	Idealized formula	Charge (mEq/g)
Cationic, 2:1 dioctahedral	Montmorillonite	$(Al_{2-x}Mg_x)Si_4O_{10}(OH)_2$ (Na_x^+, nH_2O)	0.60–1.00
	Nontronite	Fe_2^{3+} ($Si_{4-x}Al_xO_{10}$)$(OH)_2$ (Na_x^+, nH_2O)	0.76
Cationic, 2:1 trioctahedral	Hectorite	$(Mg_{3-x}Li_x)Si_4O_{10}(OH)_2$ (Na_x^+, nH_2O)	0.44
	Laponite (synthetic Hectorite)	$(Mg_{5..5}Li_{0.5})Si_4O_{10}(OH)_2$ (Na_x^+, nH_2O)	0.73
	Vermiculite	$Mg_3(Si_{4-x}Al_xO_{10})(OH)_2$ (Na_x^+, $Ca_{x/2}^{2+}$, nH_2O)	1.20–2.00
Sepiolite		$(Mg_{8-y-z}R_y^{3+}{}_3)(S_{12-x}\ R_x^{3+})(OH)_4(OH_2)_4R_{x-y+2}^{2+}$ $(H_2O)_8$	
Anionic	Hydrotalcite	$[Mg_6Al_2(OH)_{16}]CO_3.4H_2O$	3.3
	Layered Double Hydroxides (LDHs)	$[M_{1-x}^{II}M_x^{III}(OH)_2]X$, nH_2O	1.0–1.5
	[Zn_3–Al–Cl]	$[Zn_3Al(OH)_8]Cl$, $2H_2O$	2.32
	Friedel's salt	$CaO\text{-}Al_2O_3\text{-}CaCl_2\text{-}H_2O$	

double hydroxides (LHDs), also called anionic clays, display unique physical and chemical properties surprisingly close to the properties of cationic clays. These synthetic anionic clays are very promising materials for a large number of possible applications due to their versatility, easily manipulated properties, wide range of compositions, and low cost (Rives, 2001). They are also used as electrode modifiers.

In this contribution, the applications of cationic and anionic clays most relevant to electroanalysis are examined; special attention is paid to the development of biosensors based on clay-modified electrodes.

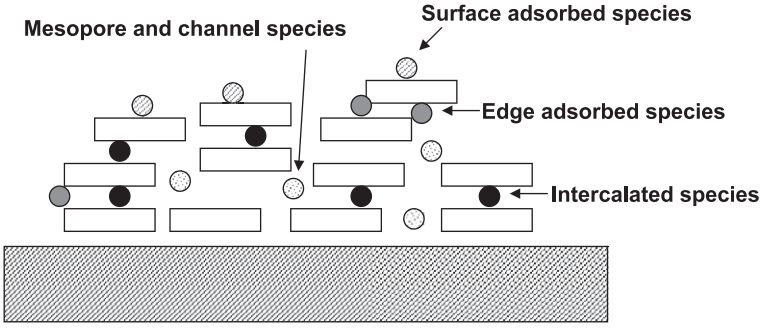
2. Electrochemistry at clay-modified electrodes

Electron transfer at clay-modified electrode (CLME) has been extensively studied. In the 1990s, several excellent reviews appeared that summarized the mechanism of electrochemistry occurring at CLME (Baker and Senaratne, 1994; Bard and Mallouk, 1992; Macha and Fitch, 1998; Therias and Mousty, 1995). Electroactive species can be accumulated within the clay layer at different places and only a small fraction of these species display redox activity (≈10–30%) (Scheme 1). Indeed, a common problem encountered when using nonconductive solids to modify electrodes is poor charge transport. The charge transport efficiency is related either to physical diffusion in the channels and/or to charge hopping. Yao et al. (1998a,b,c) investigated, by electrochemical quartz crystal microbalance (EQCM) measurements, the mass transport processes through cationic and anionic clays. Their study showed that the charge balancing during a redox reaction was accomplished by the leaching or insertion of mobile ions at the clay-solution interface. This phenomenon depends on the nature and the concentration of the electrolyte, which can cause modifications in the swelling properties of the clays.

Charge transport within the clay film can be enhanced by delamination processes that give less ordered coatings and consequently allow access to the channels. This can be achieved by different strategies, for example, by using smaller size particles (laponite instead of montmorillonite), pillaring agents (alumina, silicate) to obtain a porous clay heterostructure, or by the intercalation of molecules (surfactants or polymers). As examples, we cite the recent works of Carrero and León (2001), Falaras et al. (2000), and other references cited in the CLME preparation section.

An alternative method consists of the enhancement of electron hopping using electron relay. The strategy, developed mostly by Villemure et al., makes use of redox active cation sites within the crystal lattice (i.e., iron, cobalt, or copper for cationic clays and nickel, cobalt or manganese for anionic clays) to transfer electrons from intercalated ions to the conductive substrate (Xiang and Villemure, 1992, 1995, 1996; Qiu and Villemure, 1995, 1997; Xiao and Villemure, 1998). Other possibilities for delivering charges can be achieved by using a conductive polymer (polypyrrole) within the clay interlayer (Rudzinski et al., 1988; Faguy et al., 1994) or by using a composite conducting material (V_2O_5) (Anaissi et al., 1999).

In spite of the peculiarity of electron transfer occurring at CLME, many applications have been found for these modified electrodes, such as electrocatalysts, photocatalysts, sensors, and biosensors. It



Scheme 1. Different adsorption sites at clay-modified electrode.

should be noted that the direct electrochemistry of heme proteins (i.e., cytochrome *c*, cytochrome *P*450, myoblobin, etc.) was reported at a CLME (Lei et al., 2000; Sallez et al., 2000; Bianco, 2002; Scheller et al., 2002). The protein-clay films were generally prepared by depositing a certain concentration of protein onto the CLME. Clay modification of the electrode surface was found to facilitate the heterogeneous electron transfer process between the protein and the electrode surface. The cationic clays may hold, by electrostatic interactions, the protein in a "productive "orientation, facilitating the fast heterogeneous redox reaction with the electrode. The heme Fe(III)/(II) electroactive group of the incorporated proteins was not seriously influenced by the interaction of the proteins with the clay particles and proteins in clay films generally retained their native structures. Sallez et al. (2000) reported a detailed study on the interactions between c-type cytochromes and clays. Different types of clay were used: montmorillonite, kaolinite, and goethite according to their global charge. They confirm the role of electrostatic and hydrophobic interactions in the electrochemical promotion of c-type cytochromes systems. In the case of strong adsorption of these proteins on montmorillonite, a denaturation occurs, and consequently the metal activity is lost.

3. Clay-modified electrode preparation

The electrochemical behavior of CLME is very dependent on the method of preparation. Clay films have been cast by slow evaporation of colloidal suspensions on electrode surfaces, such as platinum, glassy carbon, indium tin oxide, and recently screen-printed electrodes (SPE). This physical adsorption, which remains the most widely used technique, has the advantage of simplicity and ease. Spin coating thin clay films and clay-carbon paste-modified electrodes have also been widely used. A more sophisticated strategy makes use of silane linkages to couple clay to the underlying electrode surface (Rong et al., 1990). Recently, the Langmuir–Blodgett method was applied to prepare thin film of clays on electrode surfaces (Hotta et al., 1997; Okamoto et al., 2000; He et al., 2001a,b). In their last paper, He et al. (2003a,b) reported that a hybrid film of chiral metal complex and a clay were prepared by the Langmuir–Blodgett method for the purpose of chiral sensing. Finally, Shirtcliffe (1999) and Song and Villemure (1999) realized electro-deposition of kaoline and montmorillonite using rotating quartz crystal microbalance disk electrodes or tin-doped In_2O_3-coated glass substrates (ITO).

4. Electroanalysis applications

Table 2 presents a compilation of the use of CLME for the electrochemical sensing of various chemicals. Table 3 focuses on biosensor applications. In both cases, a brief description of the modified electrode configuration as well as available detection limits is given.

4.1. Preconcentration methods

The preconcentration at modified electrode is analogous to trace analysis by the electrochemical technique called stripping voltammetry. The target analyte is accumulated from a dilute solution into the modified electrode layer and subsequently reduced or oxidized by a linear potential sweep (cyclic voltammetry, CV) or by a pulse potential modulation (differential pulse voltammetry, DPV; or square wave voltammetry, SWV) (Scheme 2). The detection procedure consists of the measurement of the current as a function of the potential. This approach enhances the sensitivity and the detection selectivity due to the barrier effect to opposite-charged interferents. Clays are excellent ion exchangers and their ion exchange selectivity has been applied to the accumulation of charged electroactive analytes. Preconcentration applications at CLME are summarized in Table 2. Cationic exchanging clays are essentially used for these analytical devices. The electroactivity of the adsorbed ions depends on the soaking time of the CLME in the analyte solution (accumulation time), on the nature and concentration of analyte, on the mode of preparation of the CLME, on the electrolyte nature, etc.

Table 2
Applications of clay-modified electrodes to chemical analysis

Analyte	Clay	CME configuration	Method	LoD (M)	Reference
Fe^{3+}	Montmorillonite	CPE	DPV	4×10^{-6}	Wang and Martinez, 1989
Ag^{+}	Vermiculite	CPE	DPV	model	Kalcher et al., 1995
Ag^{+}	Vermiculite	CPE	SWV	6×10^{-8}	Švegl et al., 1998
Cu^{2+}	Vermiculite	CPE	SWV	5×10^{-9}	Ogorevc et al., 1995
Cu^{2+}	Montmorillonite	CPE	DPV	4×10^{-8}	Kula and Navrátilová, 1996
Cu^{2+}	Laponite	Langmuir–Blodgett monolayer	DPV	8×10^{-5}	Barančok et al., 2002
Hg^{2+}	Vermiculite	CPE	SWV	6×10^{-8}	Švegl et al., 1998
Hg^{2+}	Monmorillonite	CPE	DPV	1×10^{-10}	Huang et al., 2002
Cd^{2+}	Bentonite	CPE	DPV		Marchal et al., 1999
Ca^{2+}	Montmorillonite	Ionophore-clay polymer composite	PISE	4×10^{-6}	Wang and Chou, 2000
$Ru(NH_3)^{3+}$	Montmorillonite	Coating/Pt	SWV	1×10^{-8}	Wieglos and Fitch, 1990
Cc^{+}, Fc^{+} derivatives	Laponite	Coating/GCE	SWV	4×10^{-8} –	Labbé et al., 1994
Methyl viologen	Nontronite	Nafion-clay composite	SWV	3×10^{-9}	Zen et al., 1996a
Methyl viologen	Laponite	Coating/GCE	SWV	5×10^{-6}	Pecorari and Bianco, 1998
Clozapine	Sepiolite	CPE	DPV	1×10^{-7}	Hernandez et al., 1988a
Flunitrazepam	Bentonite	CPE	DPV	1.3×10^{-7}	Hernandez et al., 1988b
Aniline	Sepiolite	CPE	DPV	1.6×10^{-7}	Hernandez et al., 1988c
Dinocap	Hectorite Sepiolite	CPE	DPV	1×10^{-8} 8×10^{-10}	Hernandez et al., 1988d
Tetramethrin	Sepiolite	CPE	DPV	1.4×10^{-7}	Hernandez et al., 1989
Bentazepam	Sepiolite	CPE	CV		Hernandez et al., 1990a
Linuron	Sepiolite	CPE	DPV	3.0×10^{-7}	Hernandez et al., 1990b
Ephedrine	Sepiolite	CPE	DPV	1.8×10^{-5}	Chicharro et al., 1995
Nitrobenzene	Sepiolite	CPE	DPV	1.6×10^{-6}	Lorenzo et al., 1988
Phenol	Sepiolite	CPE	DPV	3.2×10^{-7}	Hernandez et al., 1988e
2-Nitrophenol	Bentonite	CPE	DPV	1.4×10^{-7}	Rodríguez et al., 1997a,b
Nitrophenol	Montmorillonite	Coating/GCE	DPV	1×10^{-7}	Hu et al., 2001
2,4 Dichlorophenol 2,4 Dichlorophenoxyaceticacid	Hectorite	CPE	DPV	– –	Ozkan et al., 2002
2,4 Dichlorophenol	Hectorite	Clay-sol–gel/GCE Clay-sol–gel/PGE	DPV	3×10^{-8} 2×10^{-8}	Ozsoz et al., 2003
2-*sec*-butyl-4,6-dinitrophenol 2-*sec*-butyl-4,6-dinitrophenol	Sepiolite	CPE	DPV	1×10^{-10} 3×10^{-7}	Sreedhar et al., 2003
Ascorbic acid	Montmorillonite	Polypyrrole-clay composite	CV	model	Faguy et al., 1994
Dopamine Uric acid	Nontronite	Nafion/clay Coating/GCE	SWV	3×10^{-9} 2×10^{-7}	Zen and Chen, 1997
Dopamine Catechol	Nontronite	Coating/GCE	SWV	6×10^{-11} 1×10^{-10}	Zen and Chen, 1998
Dopamine	Montmorillonite	Coating/Carbon fiber microelectrode	DPV	1×10^{-8}	Liju et al., 1999

(continued on next page)

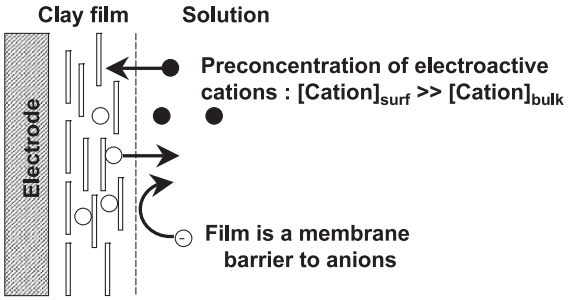
Table 2 (*continued*)

Analyte	Clay	CME configuration	Method	LoD (M)	Reference
Dopamine	Laponite	Nafion-clay composite	Chrono amperometry	5×10^{-8}	Lacroix et al., 1999
Dopamine	Nontronite	Py-clay coating/GCE	SWV	5×10^{-10}	Zen et al., 2003
Arbutin	Nontronite	Coating/SPE	SWV	2×10^{-7}	Shih and Zen, 2000
Amitrole	Nontronite	Coating/SPE	SWV	3×10^{-6}	Zen et al., 2001
Codeine	Nontronite	Coating/SPE	SWV	2×10^{-8}	Shih et al., 2002
Hypoxanthine				3×10^{-7}	
Xanthine	Nontronite	Coating SPE	SWV	7×10^{-8}	Zen et al., 2002
Uric acid				4×10^{-7}	
$[HgCl_4]^{2-}$	Montmorillonite	CPE	DPV	5×10^{-8}	Kula et al., 1999
$[HgCl_4]^{2-}$				–	
$[HgCl_3]^{-}$				–	
$[Hg(ac)_4]^{2-}$	Montmorillonite	CPE	DPV	–	Navrátilová and Kula, 2000a
$[AuCl_4]^{-}$	Vermiculite			–	Navrátilová and Kula, 2000b
$[AuCl_4]^{-}$	Montmorillonite	CPE	DPV	8×10^{-7}	Kula and Navrátilová, 2001
NO_3^-, Cl^-, SO_4^{2-}, $Cr_2O_7^{2-}$, $Fe(CN)_6^{3-}$, CH_3COO^-	Montmorillonite-chitosan nanocomposite	Nanocomposite-graphite pellets	PISE	–	Darder et al., 2003
I^-	Friedel' salt (LDHs)	CPE	DPV	6×10^{-8}	Walcarius et al., 2001
Cl^-				5×10^{-5}	
NO_3^-	[Mg–Al–X] LDHs	Coated wire electrode	PISE	2×10^{-4}	Ballarin et al., 2000a
SO_4^{2-}	[Mg–Al–X] LDHs	Polymeric-clay coating	PISE	4×10^{-5}	Morigi et al., 2001

Cationic heavy metals have been preconcentrated at CLME. Clay carbon paste electrodes (CPEs) are generally used for this application. Recently, a selective analysis of Cu^{2+} was obtained at carbon microelectrode coated with monolayers of laponite clay and polythiophene (Barančok et al., 2002). The detection limits range between 10^{-10} and 10^{-6} M, depending on the nature of the metal cations or on the clay species (Table 2). The best values reported for copper (5×10^{-9} M) and mercury (1×10^{-10} M) are lower than the European norms required for drinking water, namely 3×10^{-5} and 5×10^{-9} M (CE, 1998).

Cationic organometallic substances based on ruthenium (Wieglos and Fitch, 1990), cobaltocenium, and ferrocene (Fc) (Labbé et al., 1994) were also accumulated at CLME. The cationic species were collected in the clay films under open-circuit conditions whereas the procatonic species (ferrocene derivatives) were collected in their cationic form by applying a positive potential. Paraquat, a herbicide also known as the dicationic methylviologen (MV^{2+}), has been preconcentrated at CLME (Zen et al., 1996a,b; Pecorari and Bianco, 1998). The clay that showed the best performance is nontronite, a ferruginious smectite clay with a detection limit for MV^{2+} of 0.5 ppb (Zen et al., 1996a).

Neutral molecules such as pesticides, drugs, and purine bases have also been detected at CLME by means of adsorption stripping technique associated with differential pulse voltammetry (DPV) or square wave voltammetry (SWV) (Table 2). Carbon paste electrodes modified with sepiolite or bentonite give rise to detection limits of around 10^{-7} M. The preanodization of CLME allowed an enhancement in the current response by creating higher iron oxidation states in the interlayer of nontronite clay that resulted in a strong complexing force for the substrates; this led to a decreased detection limit in the range of 10^{-8} to 10^{-10} M (Zen and Chen, 1997, 1998; Zen et al., 2001, 2002; Shih and Zen, 2000; Shih et al., 2002). In the same way, the enhanced current in the case of Na^+-organo-hectorite was attributed to the ability of this clay to achieve electrical neutrality upon oxidation of the dichlorophenol moiety through the facile ejection of sodium ions (Ozkan et al., 2002). This amphiphilic heterostructured fluorohectoric has been shown to be an efficient



Scheme 2. Preconcentration step at clay-modified electrode.

electrochemical sensor for the specific detection of 2,4 dichlorophenol when incorporated into a carbon paste electrode (Ozkan et al., 2002) or as modifier of a glassy carbon electrode (GCE) or a single use pencil graphite electrode (PGE) (Ozsoz et al., 2003).

The anion exchange capacity of the clay minerals is about 4–5 times lower than the cation exchange capacity. In spite of this fact, an anion exchange of several complex anions $[HgCl_4]^{2-}$, $[Hg(ac)_4]^{2-}$, and $[AuCl_4]^-$ has been applied to analytical determinations of these anions (Kula et al., 1999; Navrátilová and Kula, 2000; Kula and Navrátilová, 2001). The intercalation of a cationic biopolymer chitosan in montmorillonite provides a robust nanocomposite with anionic exchanger properties (Darder et al., 2003). These materials have been successfully used in the development of bulk-modified electrodes applied to the potentiometric determination of several anions with a high selectivity towards monovalent anions such as NO_3^-, CH_3COO^-, and Cl^-.

Walcarius et al. (2001) was the first to report the use of layered double hydroxide (LDH)-modified carbon paste electrode for anodic stripping determination. A synthetic Friedel's salt was used as a carbon paste modifier for the accumulation of iodide species and their subsequent voltammetric determination. Previously, [Mg–Al–X] LDHs have been used to prepare ion sensitive electrodes for potentiometric sensing of intercalated anions species, namely sulphate, nitrate, and chloride (Ballarin et al., 2000a; Morigi et al., 2001). Another potentiometric study of the solubility of cationic surfactants has been developed based on laponite membrane electrodes (Baillarger et al., 1994).

4.2. Electrocatalysis sensors

Electrocatalysis involves an electron transfer mediation between the target analyte and the electrode surface by an immobilized catalyst, resulting in either a decrease in overpotential or an increase in peak current or both (Scheme 3). CLMEs were used to immobilize various redox mediators and applied to electroanalysis processes. As shown hereafter, the redox mediators, which are reversible electroactive species, can be immobilized within the interlayer space of the clay but can also be situated within the clay structure. A mixture of both systems was also used.

Redox mediators, i.e., hexacyanoferrate ($Fe(CN)_6^{4-}$), and cobalt phtalocyanine (CoTSPc) complexes were incorporated inside layered double hydroxides (LDHs). Carbon paste electrode modified with the resulting [Mg–Al–$Fe(CN)_6^{4-}$] was used to determine ascorbic acid (AA) within a linear range of 10^{-4} to 10^{-3} M, following the electrocatalytic scheme (Labuda and Hudáková, 1997):

$$[Mg-Al-Fe(CN)_6^{4-}] \rightleftharpoons [Mg-Al-Fe(CN)_6^{3-}] + 1e^- \quad (1)$$

$$2[Mg-Al-Fe(CN)_6^{3-}] + AA \rightleftharpoons 2[Mg-Al-Fe(CN)_6^{4-}] + DA + 2H^+ \quad (2)$$

Table 3
Biosensing applications of clay-modified-electrodes

Protein	Analyte	Mediator redox	Clay	CME configuration	LoD (M)	Reference
First generation—Oxygen based biosensors						
GOD	Glucose	–	Laponite	En-clay-GA film	1×10^{-4}	Poyard et al., 1996
GOD	Glucose	–	Laponite-PS	En-clay film	–	Coche-Guérente et al., 1998
GOD	Glucose	–	Laponite	En-clay-GA/Nafion composite	1×10^{-5}	Poyard et al., 1998
GOD	Glucose	–	Laponite	En-clay-poly(4vinylpyridine-co-styrene) composite	1×10^{-5}	Poyard et al., 1999
GOD	Glucose	$[Ru(NH_3)_6]^{2+}$	Nontronite	En-BSA-GA/clay bilayers	1×10^{-3}	Ohsaka et al., 1990
GOD	Glucose	TiO_2	Laponite	En-clay-GA film	1.5×10^{-5}	Cosnier et al., 1997b
GOD	Glucose	MV^{2+}	Montmorillonite	Clay-Sandwich configuration	5×10^{-6}	Zen and Lo, 1996
GOD	Glucose	$Ru(CN)_6^{4+}$	Montmorillonite	En-clay-BSA-GA film	1×10^{-5}	Shyu and Wang, 1998
GOD	Glucose	Luminol	Montmorillonite	En-clay-GA	1×10^{-5}	Ouyang and Wang, 1998
CO + CE	Cholesteryl oleate	–	Laponite	En-clay-polypyrrole composite	2×10^{-5}	Besombes et al., 1995
CO	Cholesterol	–	Laponite	En-clay-polypyrrole composite	–	Besombes et al., 1997
PPO	Phenol	–			–	
PPO	Catechol		Laponite-PS_2	En-clay film	5×10^{-10}	Coche-Guérente et al., 1999
PPO	Phenol		Laponite-PS_2	En-clay film	5×10^{-10}	Coche-Guérente et al., 2001
PPO	Phenols	Azure B	Laponite	En-clay-GA film	$1–17\times10^{-9}$	Shan et al., 2003c
PPO	Phenols	Polyazure B	Laponite	En-clay-GA/polymer bilayer	$0.2–4\times10^{-9}$	Shan et al., 2002
PPO	Phenols	–	Laponite [Zn–Al–Cl] LDHs	En-clay-GA film	7×10^{-10}	Shan et al., 2003b
PPO	Cyanide	–	Laponite [Zn–Al–Cl] LDHs	En-clay-GA film	1×10^{-10}	Shan et al., 2004a
MP + GOD + MR	Phosphate	–	Laponite	En-clay-GA film	1×10^{-6}	Mousty et al., 2001
XO	Hypoxanthine	MV^{2+}	Montmorillonite	En-Polyaniline/clay CPE	8×10^{-7}	Hu et al., 2000
CPE						
Second generation—Mediator based biosensors						
GOD	Glucose	TTF	Montmorillonite	En-clay-BSA-GA film	1×10^{-4}	Lei et al., 1996
GOD	Glucose	Dopamine	Nontronite	En-clay-BSA-GA film	7×10^{-6}	Zen and Chen, 1997
HRP	H_2O_2	Methylene green	Montmorillonite	En-clay-BSA-GA film	4×10^{-7}	Lei et al., 1996

HRP	H_2O_2	DHB	Laponite	En-clay-polymer composite	–	Cosnier et al., 2000
HRP + GOD	Glucose	–				
HRP	H_2O_2	ABTS	[Zn–Cr–ABTS]	En-clay-GA film	1×10^{-8}	Shan et al., 2003a
HRP + GOD	Glucose		LDHs		1×10^{-8}	
HRP	Cyanide	ABTS	[Zn–Cr–ABTS] LDHs	En-clay-GA film	5×10^{-9}	Shan et al., 2004b
Diaphorase	NADH	PolyMethylene blue	Laponite	En-clay-polymer composite	–	Cosnier and Le Lous, 1996b
LaDH	Lactate	PolyMethylene blue	Laponite	En-clay-polymer composite	1×10^{-6}	Cosnier and Le Lous, 1996a
AlDH	Ethanol				2×10^{-5}	
LaDH + Fre	Lactate	NADH and Riboflavin	Laponite	En-clay-polymer composite	1×10^{-6}	Cosnier et al., 1997a,b, p. 685
Hydrogenase	H_2	Polybutylviologen	Montmorillonite	Clay-Sandwich configuration	–	Qian et al., 2002, 2003
Third generation—Directly coupled enzyme electrodes						
Cytochrome C	H_2O_2	–	Montmorillonite	Pr-clay-colloid Pt film	1×10^{-6}	Lei et al., 1999
Hemoblobin	TCA	–	Bentonite	Pr-DDAB-clay film	model	Chen et al., 1999
Hemoblobin	TCA	–	Montmorillonite	Pr-clay film	1×10^{-6}	Fan et al., 2000
Hemoblobin	H_2O_2 NO	–	Montmorillonite	Pr-clay film	model	Lei et al., 2002
Mioglobin	H_2O_2					
Hemoblobin	O_2				3×10^{-5}	
HRP	TCA $NaNO_2$	–	Bentonite	Pr-clay film	3×10^{-5} $5\times10^{-3}(NaNO_2)$	Zhou et al., 2002
Other electrochemical biosensors						
GOD	Glucose				–	Senillou et al., 1999
Urease	Urea	–	Laponite	En-clay-GA/polymer bilayer electrodes	1×10^{-5}	
Urease	Urea Tetraborate	–	Laponite [Zn–Al–Cl]	En-clay-GA film/ISFET	2×10^{-6} 3.5×10^{-6}	de Melo et al., 2002

Similarly, [Mg–Al–CoTSPc] LDHs present electrocatalytic activity toward the oxidation of cysteine. However, Ballarin et al. (2002) did not successfully develop in analytical applications of [Mg–Al–CoTSPc]-modified electrodes.

LDHs, in which Me (II) is a transition metal undergoing a redox reaction, have been proposed to improve the charge transport of the materials. These conductive layered materials have found applications as amperometric sensors. [Ni–Al–X]-modified electrodes have been applied to the electrocatalytic detection of mono- and polyhydric alcohols (such as methanol, ethanol, propanol, glucose, fructose, sucrose, etc.) (Ballarin et al., 2000b; Scavetta et al., 2002). Polyhydric compounds characterized by a greater number of oxidable groups display higher sensitivities and narrower linear ranges.

Oyama and Anson (1986) were the first to report that the $[Ru(NH_3)_6]^{3+}$-containing montmorillonite clay coating electrocatalyzed the reduction of H_2O_2 to H_2O. Zen et al. (1996b) showed the electrocatalytic detection of hydrogen peroxide at clay-modified electrodes based on nontronite that was exchanged with methylviologen (MV^{2+}) as a redox mediator. The proposed mechanism of the catalytic reaction involves both ion-paired species of MV^{2+} adsorbed in excess of the cationic exchange capacity (CEC) and iron cations in the tetrahedral edge surface of the nontronite as follows:

$$MV^{2+} + 1e^{-} MV^{+} \quad (3)$$

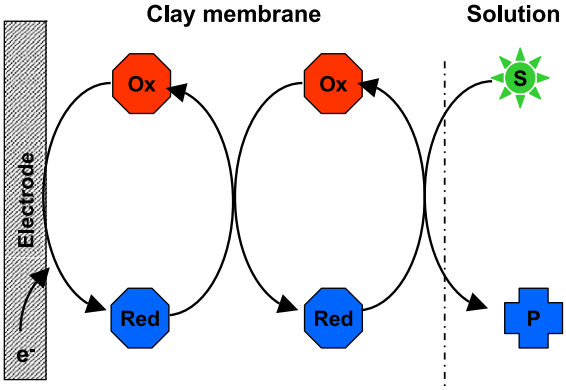
$$MV^{+} + Fe^{3+} \rightleftharpoons MV^{2+} + Fe^{2+} \quad (4)$$

$$2Fe^{2+} + H_2O_2 + 2H^{+} \rightleftharpoons 2Fe^{3+} + 2H_2O \quad (5)$$

MV^{2+} transfers an electron from the electrode surface to a fixed Fe^{3+} site within the clay, which catalyses the reduction of H_2O_2. Similarly, the electrocatalytic reduction of molecular oxygen has been reported at an iron-containing montmorillonite SWy-2-MV^{2+} carbon paste-modified electrode (Hu, 1999; Hu et al., 2000). This modified electrode offers the possibility of developing a simple and rapid analytical method for dissolved oxygen. More recently, nontronite has been converted into a more efficient catalyst by the in situ precipitation of lead ruthenate pyrochlore (Py) inside the clay host matrix (Zen et al., 2003). This new material was applied to the electrocatalytical detection of dopamine.

4.3. Amperometric biosensors

As seen in previous examples, a chemical sensor is a device that transforms chemical information into an analytical useful signal. Biosensors are chemical sensors in which the recognition system uses a biological sensing



Scheme 3. Electrocatalysis at clay-modified electrode.

element. The most popular and up to now most reliable kind of biosensors are the enzyme electrodes. The stable immobilization of an enzyme on an electrode surface, with complete retention of its biological activity and good diffusional properties for substrates, is a crucial problem for commercial development of biosensors. Various methods for enzyme immobilization are reported, such as cross-linking of proteins by bifunctional reagent, covalent binding, and entrapment in a suitable matrix. Among all the inorganic and organic matrices reported in the literature, clays occupy a privileged place due to their hydrophilic, swelling, and porosity properties. For examples, Besombes et al. (1995, 1997) have shown that the incorporation of laponite particles within an electrogenerated polypyrrole matrix strongly improves the analytical performance of amperometric biosensors based on polyphenol oxidase (PPO) and cholesterol oxidase (CO). Similarly, we have observed that laponite matrix improved the analytical characteristics and the long-term stability of biosensors compared to the corresponding biosensors simply obtained by the chemical cross linking of glucose oxidase (GOD) or polyphenol oxidase (PPO) on the electrode surface (Cosnier et al., 1997b; Shan et al., 2003c).

The entrapment of biomolecules in clay matrix constitutes an inexpensive, fast, and easy method for the elaboration of enzymes electrodes. The procedure consists of the adsorption of an enzyme/clay aqueous colloid mixture onto the electrode surface. However, the lifetime of this kind of biosensor is markedly reduced by a slow release of enzymes into solution. To overcome this problem, enzyme cross linking agents, such as glutaraldehyde (GA) (Poyard et al., 1996), bovine serum albumin (BSA) + GA (Lei and Deng, 1996), polymethyl methacrylate (PMMA), or poly(*o*-phenylenediamine) (PPD) (Shyu and Wang, 1998), have been added in the enzyme/clay coating film. This method of deposition offers the possibility of entrapping a large and well known amount of enzyme into the clay film and can be adapted not only for classical electrodes (Pt, glassy carbon electrode, transparent electrode) but also for interdigited microelectrodes (Senillou et al., 1999) or pH-sensitive field effect transistors (pH-FETs) (de Melo et al., 2002).

The clay sandwich method has also been used to immobilize glucose oxidase (GOD) (Zen and Lo, 1996; Zen et al., 1997) and hydrogenase (Qian et al., 2002). Coche-Guérente et al. (1998, 1999, 2001) adopted a polycationic organosilasesquioxane laponite (PS) clay matrix for their biosensor development. Good knowledge of the structure of the enzyme-octamer-laponite material allows them to propose an enzymatic kinetic model for the PPO amplification process. Finally, Cosnier's group developed several biosensors based on laponite-electrogenerated polymer composites (Table 3).

Examples of amperometric biosensing applications of clay-modified electrodes have been collected in Table 3. They are grouped following the three modes of detection reactions referred to as first, second, and third generation (Scheme 4).

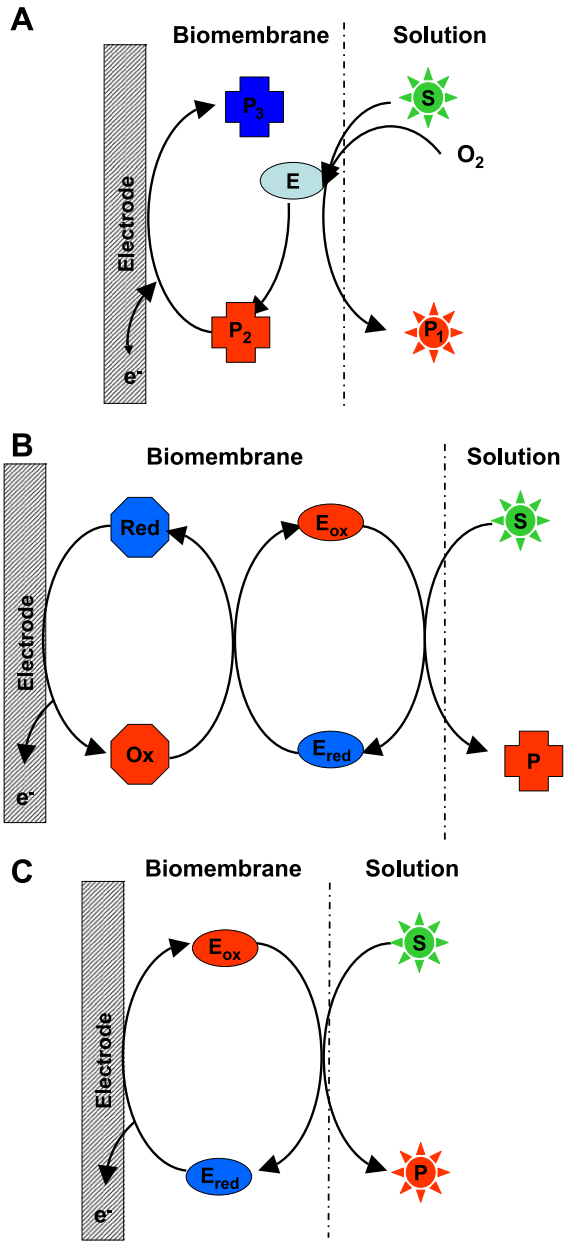
Amperometric detection of glucose dominated both analytical and fundamental studies, mainly due to the physiological importance of this analyte, the stability of glucose oxidase, and the diversity of sensing methods applied. GOD electrode used molecular oxygen as the oxidizing agent, as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2. \quad (6)$$

The amperometric detection of glucose can be carried out via the electrooxidation of the enzymatically generated H_2O_2 at a Pt electrode:

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (7)$$

Due to the high polarizing voltage applied ($E_{app} \approx 0.6$–0.8 V), interferents such as ascorbic acid and uric acid, which are commonly present in biological fluids, can also be oxidized leading to nonspecifical signals. Several attempts have been made to overcome this problem. Poyard et al. (1998, 1999) used clay-semipermeable polymer composite electrodes to decrease the permeability to organic interfering compounds. Another possibility consists of decreasing the electrode potential using redox mediators. As previously shown in the electrocatalysis part, hydrogen peroxide can be reduced by redox mediators immobilized within CLME. Such a concept was applied to



Scheme 4. Amperometric biosensors: (A) first generation, (B) second generation, (C) third generation.

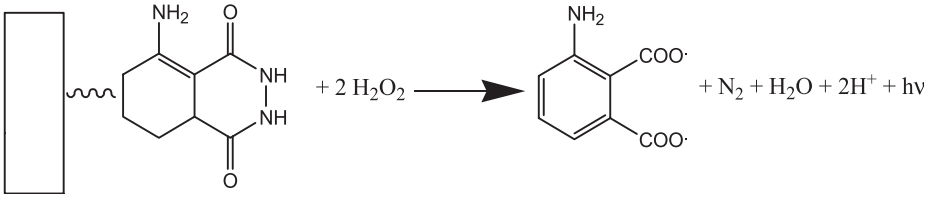
glucose biosensors based on CLME using cationic MV^{2+} (Zen and Lo, 1996), ruthenium complexes (Ohsaka et al., 1990; Shyu and Wang, 1998) associated to structural iron cations, or using TiO_2 underlying films (Cosnier et al., 1997b).

The actual oxidation of glucose is carried out by the flavin-adenine dinucleotide (FAD) component of the GOD, which is converted into $FADH_2$. After glucose oxidation, $FADH_2$ returns to FAD in the presence of oxygen (Scheme 4A). In the second generation of biosensors, the redox mediators (i.e., TTF or dopamine) were used as

oxidizing agents instead of O_2 (Lei and Deng, 1996; Zen et al., 1997). The current flowing through the re-oxidation of electron transfer agents at the electrode surface is therefore the amperometric measure of glucose concentration (Scheme 4B). Similarly, bienzymatic configurations, composed of glucose oxidase, horseradish peroxidase (HRP), and redox mediators coated on CLME, were also applied to the detection of glucose at 0.0 V preventing any possible oxidation of ascorbate and ureate (Cosnier et al., 2000; Shan et al., 2003a). All these examples showed that mediated determination of glucose can be performed at clay-modified electrodes with good sensitivities and with no interferences.

In addition to the previously described amperometric biosensors, it should be noted that a peculiar GOD/luminol/clay electrode was constructed for the detection of glucose by means of electrochemiluminescence (Ouyang and Wang, 1998). The proposed sensing mechanism is summarized as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \quad (8)$$



(9)

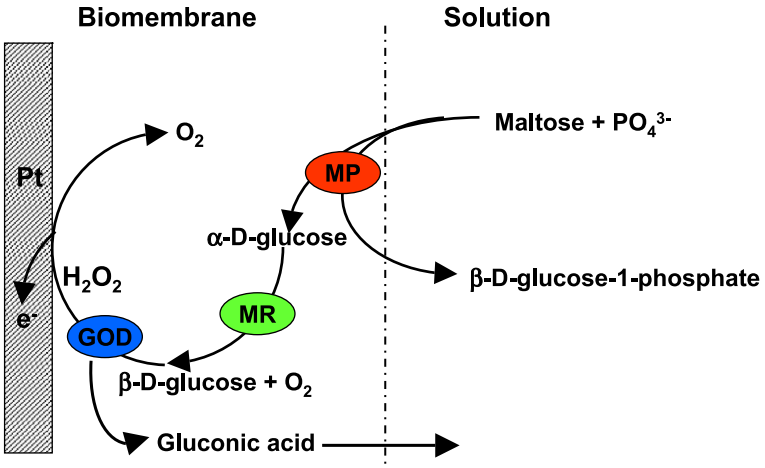
Finally, the immobilization of GOD on microelectrode arrays and their use for successful amperometric as well as conductimetric detection of glucose illustrate well the potential of enzyme/clay coating for the fabrication of conductimetric microbiosensors (Senillou et al., 1999).

Concerning hydrogen peroxide, its enzymatic detection has been reported either at mediated horseradish peroxidase (HRP) CLME or by direct enzyme regeneration at CLME (3rd generation, Scheme 4C) (Table 3). In the former case, the redox mediators methylene green (MG), poly 3,4 dihydroxybenzaldehyde (DHB), and 2,2′ -azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) were co-immobilized within the clay matrix and played the role of electron shuttle between the redox center of HRP and the electrode (Lei et al., 1996; Cosnier et al., 2000; Shan et al., 2003a). In particular, synthetic redox active [Zn–Cr–ABTS] LDHs appeared to be efficient immobilization matrices for HRP wiring. This biosensor attained a better detection limit (10 nM) for H_2O_2 compared to those obtained with other HRP biosensors based on clay or composite sol–gel matrices. On the other hand, direct electron transfer of heme proteins (HRP, myoglobin, hemoglobin, cytochrome *c*) showed potential applications in sensors for the determination of H_2O_2, as well nitrite, NO, and trichloroacetic acid (TCA) (Chen et al., 1999; Lei et al., 1999, 2002; Fan et al., 2000; Zhou et al., 2002).

Several other oxidase enzymes have been immobilized on CLME, in particular using laponite as immobilization matrix. These biosensors have been used to detect NADH, lactate, ethanol, and also a wide variety of water pollutants, such as phenols, cyanide, and phosphate (Table 3).

Cosnier and Le Lous (1996a,b) presented a biosensor configuration based on the entrapment of diaphorase or dehydrogenase within laponite gel containing an electropolymerized mediator (Methylene blue, MB) for NADH oxidation. Poly MB in the biolayer allowed an electron transfer communication between enzymes and the electrode surface. This Poly MB/dehydrogenase/laponite-modified electrode was successfully used for electro-enzymatic detection of lactate and ethanol via the mediated oxidation of NADH at 0 V.

A biosensor for phosphate detection has been fabricated by the co-immobilization of three enzymes with complementary activities following the sequence illustrated in Scheme 5. The amperometric detection corresponded to H_2O_2 oxidation.



Scheme 5. Phosphate biosensors based on a trienzymatic clay-modified electrode.

Different configurations of PPO biosensors based on CLME have been developed in our group, giving rise to very sensitive (subnanomolar) determination of phenol derivatives (Table 3). Major drawback inherent to this type of biosensors is generally their limited lifetime due to electrode fouling induced by polymerization of unstable *o*-quinone, the product of the enzymatic reaction. Azure B and polyazure B film acted as redox mediators and enabled the mediated reduction of *o*-quinone preventing any electrode fouling. Consequently, the operational stability of the PPO/laponite electrode was greatly enhanced (Shan et al., 2002, 2003c).

Very recently, we have described a comparative study between the properties of two different biosensors based on the immobilization of PPO within the two different kinds of clay matrices: one cationic (laponite) and the other anionic (LDHs) (Shan et al., 2003b). The PPO/[Zn–Al–Cl] LDHs biosensor showed remarkable properties such as high sensitivity and good storage stability. The attractive analytical characteristics of LDH-based biosensors were certainly due to the high permeability and the structure and charge of the particles.

A large percentage of environmental pollutants are known to act as enzyme inhibitors, resulting in the development of sensors based on the measurement of this property. From a general point of view, there is no work described in the literature using clay-entrapped enzymes for inhibition biosensor applications. We have developed this concept, for the first time, in particular for cyanide determination at PPO and HRP biosensors based on LDHs' immobilization matrix (Shan et al., 2004a,b). It should be noted that the PPO/[Zn–Al–Cl] biosensor is more sensitive to cyanide traces (0.1 nM) than conventional procedures based on spectroscopic or chromatographic methods. Enzyme immobilization in this anionic exchanging clay seems to cause an increase in cyanide inhibition effects because of anion accumulation in the clay matrix.

Hu et al. (2000) have developed a novel hypoxanthine sensor based on xanthine oxidase (XO) immobilized within a polyaniline film. The detection is based on oxygen consumed due to the enzymatic reaction measured at a montmorillonite-MV^{2+} carbon paste-modified electrode.

Finally, urea biosensors have been developed by the immobilization of urease within the two different kinds of clay matrices: laponite and [Zn–Al–Cl] LDHs. The transduction process was performed either by conductimetric measurement with interdigited microelectrodes (Senillou et al., 1999) or by potentiometric measurements with pH-FETs (de Melo et al., 2002).

5. Conclusion

Clays offer attractive properties in designing electrode surfaces for analytical applications due to their stability and low cost. Ion exchange and adsorption properties have been extensively applied not only to potentiometric and amperometric determinations of heavy metals, pesticides, and drugs, but also to biosensor development. Clay-modified carbon paste electrodes have generally been used for adsorptive stripping sensors. A new alternative is clay-modified screen-printed electrodes (SPE). SPE technology offers the advantages of being inexpensive, simple, and rapid, and allows mass production of reproducible electrodes. Another promising method to prepare electrochemical sensors consists of the preparation of single layer clay film by the Langmuir–Blodgett method.

Concerning the clay nature, clay nanocomposites and synthetic layered double hydroxides are materials of increasing interest because of their structural or functional behavior. For instance, organic-inorganic hybrid material, like polymer-clay nanocomposites, presents properties inherent to both types of components. LDHs also constitute promising materials for a large number of electroanalytical applications due to their versability, their wide range of compositions and preparation variables that can readily modulate their adsorption behavior, and electrocatalysis properties in regards to analytes and/or enzymes. Exciting future prospects include the use of these synthetic materials to single-use sensors and biosensors.

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