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Electrochemical Methods for the *In Vitro* Assessment of Drug Metabolism

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1. Introduction

The development of new chemical compounds with promising pharmacological activities is a very time-consuming and costly process because it requires many studies and evaluations prior to obtaining final approval for use in humans. Thus, for the safe use of any new drug, it is necessary to first know its physical, chemical and biological properties, as well as its efficacy, stability, pharmacokinetic properties (i.e., absorption, distribution, metabolism and excretion) and toxicity.

Usually, the toxicity of a new drug or of its metabolites is the main reason most candidate drugs are removed from the development process and are not approved for human use. For this reason, it is very important to determine in the early development stages the tendency of a drug of interest to undergo bioactivation into toxic metabolites and, additionally, the reactivity of these metabolites toward biomolecules (Baumann & Karst, 2010). Therefore, an ideal drug metabolism study should include a complete identification of all metabolites generated and their bioactivation pathways, in addition to the reactions between the drug and its metabolites with endogenous molecules or xenobiotics. For this purpose, different *in vitro* and *in vivo* experimental models are commonly applied prior to clinical trials, which include the use of enzymes, liver cells, liver cell extracts and laboratory animals, among others (Brandon et al., 2003).

Rat and human liver microsomes (RLM and HLM) are the most widely used cell extracts for studying metabolic reactions because they contain high concentrations of the cytochrome P450 enzymatic complex (CYP), which catalyzes the majority of oxidative reactions in organisms (Brandon et al., 2003). However, the use of conventional *in vitro* and *in vivo* methods makes it very difficult to detect the formation of reactive metabolites or intermediates with short half-lives because these short-lived species tend to covalently bond to cellular macromolecules, such as proteins and DNA (Lohmann & Karst, 2007). For this reason, the early 1980s ushered in the development and application of new methods for studying drug metabolism. In this context, the use of electrochemistry, a purely instrumental technique, has emerged as an interesting alternative to generate and detect metabolites in the drug development process because it can mimic the predominant redox reactions that occur in the human body (Álvarez-Lueje & Bollo, 2010; Jurva et al., 2003).

Electrochemistry is useful for the study of metabolism, as it has certain advantages compared to conventional methods. Namely, it allows the generation and direct identification, in a rapid and clean manner, of both stable species and metabolites with short half-lives. Consequently, electrochemistry can be a powerful tool in interaction studies with specific cellular components, which is more difficult to realize with conventional *in vivo* or *in vitro* methods. Additionally, electrochemistry is a cost-effective, rapid and clean system that does not require the use of laboratory animals or organ extracts.

Different electrochemical methods have been applied to mimic drug metabolism and the reactivities of metabolites toward biomolecules, but the most frequently used system consists of an electrochemical cell coupled to a mass spectrometer (EC-MS). A modification of this system has involved the incorporation of a chromatographic separation between the cell and the detector, which permits on-line electrochemistry-liquid chromatography-mass detection (EC-LC–MS) (Lohmann et al., 2010). To improve this system and obtain more information about metabolic reactions, different modifications have also been utilized. For example, the incorporation of trapping agents (i.e., glutathione (GSH) or other thiols) that can form stable adducts with the reactive intermediates generated in the electrochemical cell is commonly used to detect the interactions of metabolites (Lohmann & Karst, 2006; van Leeuwen et al., 2005; Lohmann & Karst, 2009). Other electrochemical applications useful for metabolic studies include the development of enzyme-based biosensors, antibodies and DNA, among others (Joseph et al., 2003).

In the following sections, the most important electrochemical aspects that can help explain how metabolic reactions can be emulated using different electrochemical methods and the advantages of these over conventional methods are presented. Additionally, the main metabolic pathways that can be electrochemically emulated and comparisons with *in vivo* and *in vitro* assays are summarized and discussed. Finally, both the application of electrochemical tools for studying interactions between metabolites and biomolecules and the application of biosensors in this field are presented.

2. General aspects of electrochemistry

Electrochemistry involves the study of chemical phenomena associated with the separation and transfer of charge that occurs at the interface of an electrode and solution. Electrochemistry is applicable in areas ranging from basic studies to environmental and clinical applications (Speiser, 2007).

Electron transfer causes the reduction or oxidation of a substance when a sufficiently negative or positive potential is applied, respectively. As the potential values can be controlled externally, redox processes of interest can be carefully chosen, making electrochemistry useful for studying the redox behavior of any substance, inorganic or organic, including drugs, toxics, biomolecules and metabolites.

Commonly, measurements are realized in an electrochemical cell using a system of three electrodes, the working, reference and auxiliary electrodes, immersed in a solution containing the compound of interest and a supporting electrolyte. The working electrode is usually constructed from inert materials, such as allotrope derivatives of carbon (graphite or glassy carbon) or metals (mercury, platinum or gold). Reference electrodes provide a fixed potential against which the potential applied to the working electrode is measured and

controlled, and they often consist of a saturated calomel electrode or $Ag/AgCl_{(sat)}$. A third electrode, the auxiliary electrode, is often included, which normally consists of a platinum wire. Because the reference electrode has a constant potential value, any change in the electrochemical cell is due to the working electrode and the redox process. The three electrodes are connected to a potentiostat, which controls the potential applied to the working electrode, and the results are recorded by a computer (Bard & Faulkner, 2001; Brett & Oliveira Brett, 1993). A schematic representation of this system is shown in figure 1A.

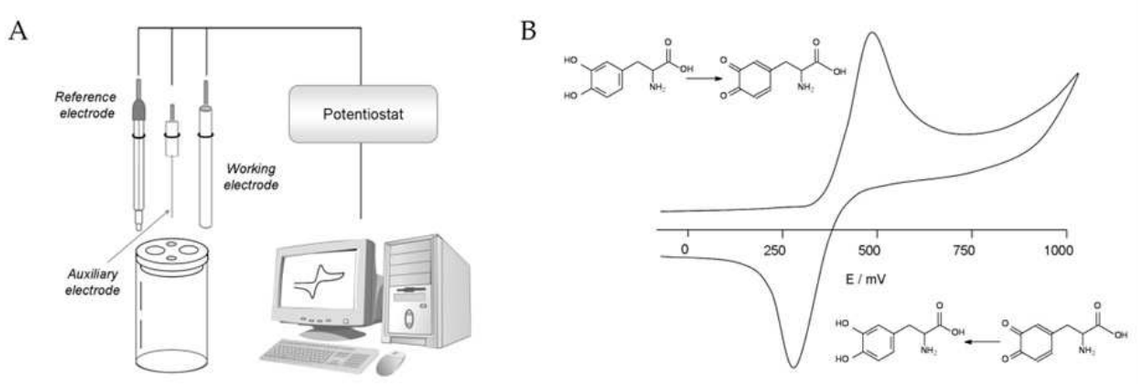


Fig. 1. A) Electrochemical cell with a three-electrode system. B) Typical redox couple obtained using cyclic voltammetry on levodopa and the respective oxidative and reductive reactions.

Different techniques are often employed in electrochemistry for the study of electroactive substances. These include linear sweep, differential pulse and cyclic voltammetry, chronoamperometry, chronocoulometry and bulk electrolysis, among others. All of these techniques can provide important information about substances of interest and the characteristics of their redox processes, but voltammetric and electrolytic methods are more relevant to the study of electroactive drugs, their metabolites and the metabolites' interactions with biomolecules.

Voltammetric methods are based on measuring changes in current by applying a fixed potential, or a potential sweep, onto the working electrode. According to the potential applied, the compound under study is oxidized or reduced at the electrode surface, while the bulk of the solution remains unchanged. Voltammetric measurements are two-dimensional, where the potential is related to the qualitative properties of the substance, and the current is related to its quantitative properties.

In linear sweep and pulse voltammetry, the potential sweep is applied in one direction only. However, the cyclic voltammetry technique makes successive potential sweeps in both directions, i.e., oxidation and reduction, between two potential values. A typical cyclic voltammogram is shown in figure 1B. Thus, cyclic voltammetry is useful for the study of both the redox properties of a substance and the behavior of the product generated during the electrochemical reaction. The applicability of this technique mainly depends on the number of compounds that are electroactive in the potential range applied, among other parameters. One highlight of cyclic voltammetry is that the rate of experiments can be regulated and, as a result, can be rapidly performed, allowing the study of species with short half-lives.

When the controlled potential electrolysis technique is used, a fixed potential is applied to the working electrodes, which have large surfaces so that the entire bulk solution can be oxidized or reduced. As a result, measurable amounts of compounds that are identifiable by other techniques, e.g., mass spectrometry, can be obtained. Thus, electrochemistry coupled to mass spectrometry (EC-MS) permits the reproduction of the redox reactions involved in drug metabolism, allowing the detection of final products or stable intermediaries (Lohmann et al., 2010).

Taking into account that electrochemistry allows investigations of the redox reactions that drugs undergo and that phase I metabolism in the liver occurs via redox reactions, electrochemical methods have emerged as a promising alternative and a complementary tool in the study of *in vitro* metabolism because they are relatively simple, fast and cost-effective systems.

3. *In vitro* mimicry of metabolic reactions by electrochemistry

The electrochemical oxidations of organic compounds and the use of electrochemistry to mimic biologic reactions, such as oxidative drug metabolism, have been well documented and revised (Álvarez-Lueje & Bollo, 2010; Jurva et al., 2000, 2003). Electrochemistry has been used to emulate different phase I reactions, such as aromatic hydroxylation, dehydrogenation and O- and N-dealkylation, by introducing the compound of interest into an electrochemical cell and applying a specific potential for a determined period of time. During this time, the entire sample is oxidized to generate one or more products, depending on the applied potential. Thus, from this method, the generated products can be identified, characterized and studied separately. Furthermore, the simulation of phase II reactions, including conjugation with GSH or other thiols from electrochemically generated phase I metabolites, has also been achieved. For example, the metabolism of clozapine, including both phase I and phase II reactions, has successfully been mimicked (van Leeuwen et al., 2005), as well as the detoxification of acetaminophen (Lohmann & Karst, 2006) and the conjugation of diclofenac (Madsen et al., 2008a) by the electrochemical oxidation of the parent drugs.

The replication of drug metabolism using electrochemistry is a faster, more cost-effective instrumental technique than the widely used *in vivo* and *in vitro* methods that utilize liver cells or isolated enzymes. On-line EC-MS has been introduced as an alternative for early-stage metabolite discovery, and its ability to mimic biological oxidation patterns is currently being explored because it allows both the oxidation of a drug and the identification of the oxidized species. In this on-line system, the sample flows through an electrochemical cell that normally contains a working electrode made of porous glassy carbon, which posses a great area. On the working electrode occurs the quantitative conversion of the tested compound, depending on the working conditions, such as the nature of the analyte, the potential applied, the flow rate and the pH. Especially in high-throughput screening, EC-MS is superior to the conventional methods, as it can be completely automated. Because of these features, on-line EC-MS is of particular importance to the pharmaceutical industry. Although the EC-MS results are not completely transferable to the conditions of the human liver, this methodology can provide primary data concerning the metabolism of drugs in the human body. Thus, the instrumental method EC-MS may have advantages or may be complementary to the existing methods of screening that involve *in vitro* studies or organ

fractions, e.g., microsomes, hepatocytes and liver slices. Additionally, with an on-line EC-MS system, the complexity of a biological system is reduced, and the optimization of the electrochemical and MS parameters can be realized more rapidly. The ternary combination of EC-LC-MS is able to detect reaction products with lifetimes on the order of several minutes; therefore, short-lived intermediates are not detected, as they react to more stable products during the chromatographic run. Direct EC-MS allows the detection of less stable compounds with lifetimes of a few seconds (van Leeuwen et al., 2005). In figure 2, a schematic representation of both systems is shown.

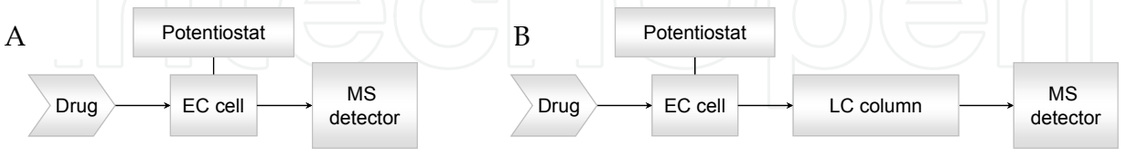


Fig. 2. Schematic representation of different on-line electrochemical set-ups used for the study of drug metabolism reactions. A) EC-MS system; B) EC-LC-MS system (adapted from Baumann & Karst, 2010).

The use of on-line EC-MS began in the early 1970s. Early studies included the development of a thin-layer electrochemical flow cell that was employed in combination with thermospray mass spectrometry for the study of the oxidation of uric acid and 6-thioxanthine (Volk et al., 1989, 1992). An analogous array in a flow injection experiment was used to study the formation of GSH and cysteine conjugates of acetaminophen. Later, the pattern of coupling an electrochemical flow cell to mass spectrometry was extended to other ionization techniques, e.g., fast-atom bombardment, particle beam, atmospheric-pressure chemical ionization and electrospray (Baumann & Karst, 2010; Jurva et al., 2000; Lohmann et al., 2008).

The first coupling of electrochemistry to particle-beam mass spectrometry was applied for the study of the oxidation pathway of dopamine (Regino & Brajter-Toth, 1997). Later, different electrochemical cells were coupled to an electrospray mass spectrometer to study the different products of biologically relevant redox reactions (Zhou & Van Berkel, 1995). In addition, an on-line electrochemistry-electrospray ionization mass spectrometry system using a microflow electrolytic cell was developed and applied to study the electrochemical oxidation of caffeic acid (Arakawa et al., 2004).

The first example of the application of EC-LC-MS for the study of drug metabolism was reported in 1989 for comparing the enzymatic and electrochemical oxidation pathways of uric acid. This study found that the enzymatic and chemical reactions yielded the same intermediates and products of those observed in the electrochemical oxidation. A similar array was used to oxidize 3-hydroxy-*dl*-kynurenine (a tryptophan metabolite) in an electrochemical cell, eventually separating the oxidation products by reversed-phase LC and identifying the products by UV and MS detection (Deng & Van Berkel, 1999).

Comparisons on the mechanistic level have been made for most reactions to explain why certain reactions can and other reactions cannot be mimicked by electrochemical oxidations. The EC system successfully mimics metabolic pathways in cases where the CYP-catalyzed reactions are supposed to proceed via a mechanism initiated by one-electron oxidation, such as N-dealkylation, S-oxidation, P-oxidation, alcohol oxidation, dehydrogenation and

hydroxylation of aromatic rings that contain electron-donating groups. In contrast, the CYP-catalyzed reactions initiated via direct hydrogen abstraction, such as O-dealkylation, aliphatic hydroxylation and hydroxylation of aromatic rings without electron-donating groups, generally have a very high oxidation potential for electrochemical oxidation and cannot always be mimicked using EC (Johansson et al., 2007). However, even when the EC system is not able to mimic all oxidations performed by CYP, valuable information can be obtained regarding the sensitivity of the substrate toward oxidation and the molecular position where oxidation is likely to occur.

For the reason described above, to be able to mimic all CYP-catalyzed oxidations, electrochemistry must be complemented with other oxidative systems. This is the case for the electrochemically assisted Fenton reaction (EC-Fenton) that is able to mimic aliphatic hydroxylation, benzylic hydroxylation, aromatic hydroxylation, N-dealkylation, N-oxidation, O-dealkylation, S-oxidation and dehydrogenation (Johansson et al., 2007). In EC-Fenton, the regeneration of Fe^{+2} is achieved by the reduction of Fe^{+3} at the working electrode. The hydroxyl radical generated from this system is highly electrophilic and readily reacts with aromatic rings and double bonds. In addition, the hydroxyl radical can abstract a hydrogen atom from various organic compounds.

Studies on drug metabolism by electrochemical techniques will be presented, discussed and compared with conventional *in vitro* (e.g., microsomes, hepatocytes and porphine system) and *in vivo* assays and divided by each main representative chemical functional group, including aliphatic hydroxylation; aromatic hydroxylation; N-, S- and O-dealkylations; heteroatom (N, S and P) oxidation; dehydrogenation; and other oxidative reactions, such as the oxidations of alcohols, aldehydes and benzylic groups. In addition, examples of drug metabolism mimicked by EC are summarized in table 1.

Drug	Predicted metabolism	Products	Urine	Plasma	RLM	HLM	Rat Bile	EC	References
Acetaminophen	Phases I, II	N-acetyl-*p*-benzoquinoneimine				√		√	Lohmann & Karst,2006;
		p-benzoquinoneimine-GSH				√		√	Madsen et al., 2007
Albendazole	Phase I	albendazole sulfoxide		√#	√	√		√	Galvão de Lima et al., 2003
		albendazole sulfone		√#	√	√		√	
Amodiaquine	Phases I, II	N-deethylated quinoneimine			√	×		√	Johansson et al., 2007; Johansson et al., 2009; Lohmann & Karst,2007; Madsen et al., 2007
		Quinoneimine			√	√		√	
		Aldehyde			√	×		√	
		GSH adducts			√	√		√	
		N-deethylation			√	√		√	
Boscalid	Phases I, II	hydroxylation, dehydrogenation	√*					√	Lohmann et al., 2009
		GSH adducts	√*					√	
Chlorpromazine	Phase I	*N*-demethylation				√		√	Nozaki et al., 2006
		S-oxidation				√		√	
		N-oxidation				√		×	
		aromatic hydroxylation				√		×	

Drug	Predicted metabolism	Products	Urine	Plasma	RLM	HLM	Rat Bile	EC	References
Clozapine	Phases I, II	N- dealkylation			√	√		√	Dain et al., 1997; Schaber et al. 2001; van Leeuwen et al., 2005;
		N-oxide	√#√#		√	√		×	
		aromatic	√#		×	×		√	
		hydroxylation	√#		√	√		√	
		GSH adducts							
Diclofenac	Phases I, II	4'-OH- diclofenac			√	√	√	√	Madsen et al., 2008a
		5-OH-diclofenac			√	√	√	√	
		GSH adducts			√	√	√	√	
Estradiol	Phases I, II	2-hydroxyestradiol			√	√		√	Gamache et al., 2003
		4-hydroxyestradiol			√	√		√	
		GSH adducts			√	√		√	
7-ethoxycoumarin	Phase I	O-dealkylation			√	√	√	√	Johansson et al., 2007; Jurva et al., 2000;
Lidocaine	Phase I	N- dealkylation			×	√	√	√	Baumann & Karst 2010; Johansson et al., 2007; Jurva et al., 2003;
		N-oxidation			√	√	×	√	
Mephenytoin	Phase I	dydroxylation			√	√		√	Johansson et al., 2007
S-methylthiopurine	Phase I	S-oxidation			√	√		√	Johansson et al., 2007
		S-demethylation			√	√		√	
Metoprolol	Phase I	benzylic hydroxylation			√	√		√	Baumann & Karst 2010; Johansson et al., 2007
		aromatic hydroxylation			√	×		√	
		N-dealkylation			√	√		√	
		O-dealkylation			√	√		√	
Mitoxantrone	Phase I	quinone			√			√	Lohmann & Karst, 2007
Parathion	Phase I	phosphine oxide	√*					√	Jurva et al., 2003
Procainamide	Phase I	N-monodealkyl procainamide			×	√		√	Odijk et al., 2010
		N-hydroxylamine procainamide			√	√		√	
		N-oxide derivative procainamide			√	×		√	
2-(N-propyl-N-2-thienylethyl amino) -5-hydroxitetralin	Phase I	N-dealkylation			√			√	Jurva et al., 2000
		hydroxylation			√			√	
Testosterone	Phase I	aliphatic hydroxylation			√	√		√	Johansson et al., 2007
Tetrazepam	Phase I	cyclohexenyl hydroxylations	√#		√			√	Baumann et al., 2009
		3-hydroxytetrazepam	×#		√			√	
		diazepam	√#		√			√	
		nortetrazepam	√#		√			√	
Toremifene	Phases I, II	N-demethylation			√	√		√	Lohmann & Karst, 2009
		N-oxide			√	√		×	
		quinone methide			√	√		√	
		GSH adducts			√	√		√	

Drug	Predicted metabolism	Products	Urine	Plasma	RLM	HLM	Rat Bile	EC	References
Triclocarban	Phase I	2′-hydroxytriclocarban							Baumann et al., 2010
		6-hydroxytriclocarban	√#	√*#	√	√	√	√	
		3′-hydroxytriclocarban	√#	√*#	√	√	√	√	
		2′,6-dihydroxytriclocarban	√#		√	√		√	
			√*		×	×		√	
		3′,6-dihydroxytriclocarban			×	×		√	
Troglitazone	Phases I, II	*p*-quinone derivative		√#	×	√		√	Madsen et al, 2008b; Tahara et al., 2007; Tahara et al., 2009
		o-quinone methide		×#	√	√		√	
		o-quinone methide conjugates			√	√		√	
Zotepine	Phase I	*N*-demethylation				√		√	Nozaki et al., 2006
		S-oxidation				√		√	
		N-oxidation				√		×	
		aromatic hydroxylation				√		×	

Table 1. Examples of drug metabolism mimicked by EC (√: generated; ×: not generated,*rat, #human).

Aliphatic hydroxylation

One limitation of the electrochemical method has been the difficulty in mimicking the metabolic hydroxylation of alkanes and alkenes due to the fact that electrochemical oxidation potentials for aliphatic hydrocarbons are generally very high (Jurva et al., 2003). Nevertheless, there have been some examples where this experimental limitation has been overcome, as in the oxidation of the muscle relaxant tetrazepam. In this case, a platinum working electrode was used, with an enhanced potential range of up to 2000 mV, instead of the typical porous glassy carbon working electrode, and the hydroxylation of the tetrazepam cyclohexenyl group at five different positions was successfully achieved (compounds 2, 3, 6, 8 and 10 in figure 3). In this study, an on-line EC-LC-MS system was used to analyze the metabolic pathway of the drug. Additionally, a comparison of the EC method with urine analyses and a conventional rat liver microsomal approach was performed, which found that eleven different metabolites were indentified in the urine samples, and only two of them were not found after microsomal incubation (table 2). These differences were explained as a result of different CYP isoforms with different catalytic activities in each organism; therefore, the metabolism in humans and in rat liver microsomes may result in different metabolites or a different distribution of them. As can be seen in this example, the comparison of both methods shows that electrochemistry combined with LC-MS is an adequate complementary tool to conventional *in vitro* methods in the prediction of metabolic processes of tetrazepam (Baumann et al., 2009).

A second example of aliphatic hydroxylation is testosterone, in which two different hydroxylation products were found in liver microsomes, one in RLM and the other in HLM. Both metabolites were generated using the EC-Fenton system and the porphine system (Johansson et al., 2007).

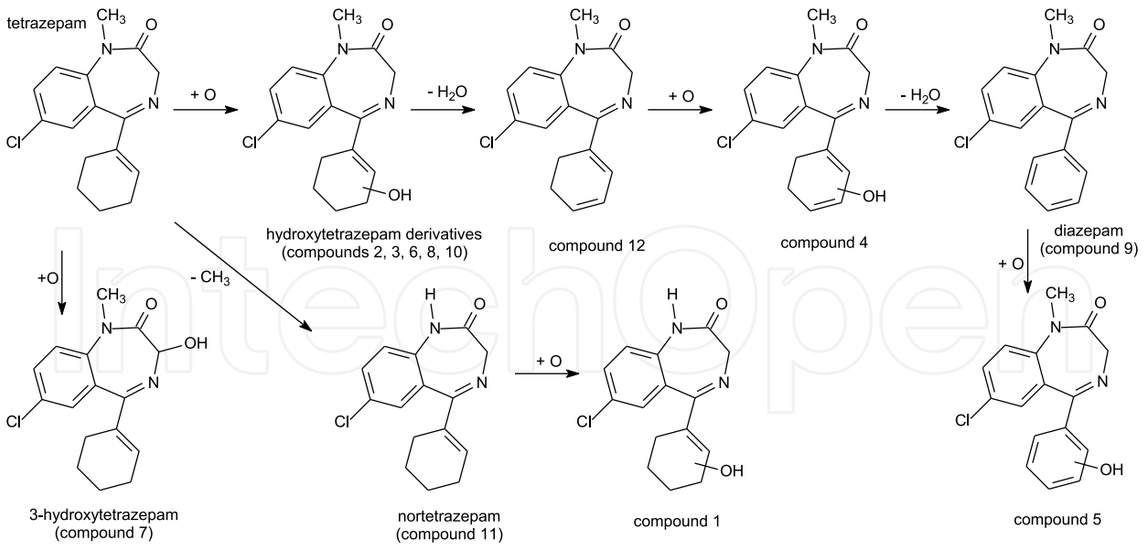
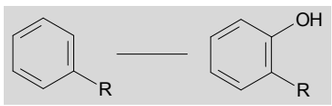


Fig. 3. Oxidative metabolism of tetrazepam (adapted from Baumann et al., 2009).

	Compound											
	1	2	3	4	5	6	7	8	9	10	11	12
Urine	x	x	x	x	x	x		x	x	x	x	x
RML	x	x	x	x			x	x	x	x	x	x
EC		x	x		x	x	x	x	x	x	x	x

Table 2. Tetrazepam metabolites produced in different systems. For compound identification, see figure 3 (adapted from Baumann et al., 2009).

Aromatic hydroxylation



Contrary to aliphatic hydroxylation, a variety of drugs and compounds have been tested for aromatic hydroxylation using EC-MS, including coumarin, *p*-nitrophenol, diclofenac, xanthohumol, tyramine and N,N-dimethylaniline (Jurva et al., 2003).

In general, to be oxidized electrochemically within the potential limits of water, the aromatic ring must be activated by an electron-donating group (e.g., a hydroxyl or amino group). For an aromatic ring without an electron-donating group, the starting compound is more difficult to oxidize than the product. For example, benzene is oxidized at a potential approximately 1300 mV more anodic than phenol. Furthermore, the oxidation cannot be stopped at the phenol stage but instead proceeds immediately to benzoquinone (figure 4). The hydroxylation of aromatic compounds by CYP shows some mechanistic similarity to electrochemical oxidation. However, due to the lack of an active iron-oxygen species in the electrochemical system, the radical intermediate achieved after the initial oxidation and deprotonation steps will react further in different pathways, which are determined by the specific substituents (Jurva et al., 2003).

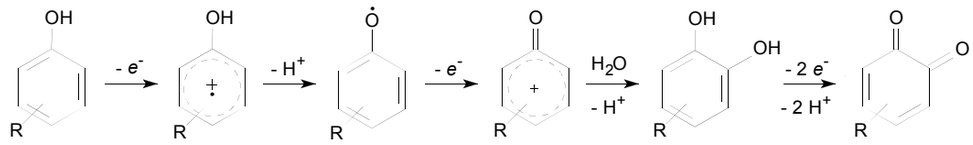


Fig. 4. Electrochemical oxidation mechanism of phenols.

The suggested mechanism for the electrochemical oxidation of phenols (figure 4) is initiated in the same way as the CYP-catalyzed oxidation. Compounds that are easily electrochemically oxidized likely follow this pathway for CYP-catalyzed oxidation, while compounds with high oxidation potentials are more likely to proceed via one of the other pathways after oxidation by CYP. In an electrochemical system, the nature of the π-system, the physical features of the working electrode and the nature of the supporting electrolyte will determine the final compound. Thus, different substituents on the aromatic ring frequently result in different products, e.g., phenols are generally oxidized to quinones, while other substituents on the aromatic ring will lead to different products.

The beta blocker metoprolol and the antiepileptic mephenytoin are good examples of compounds in which aromatic hydroxylation has been investigated. Metoprolol contains an aromatic ring with oxygen as an electron-donating group. In contrast, mephenytoin does not contain any strong electron-donating groups, and it represents an aromatic ring with lower electron density. The aromatic hydroxylation of metoprolol has been studied in RLM, and it was also simulated using a conventional EC system, the EC-Fenton system and the porphine system (figure 5). A different situation occurred with mephenytoin, whose aromatic hydroxylation has been investigated in both RLM and HLM but could only be mimicked using the EC-Fenton system and the porphine system (Johansson et al., 2007).

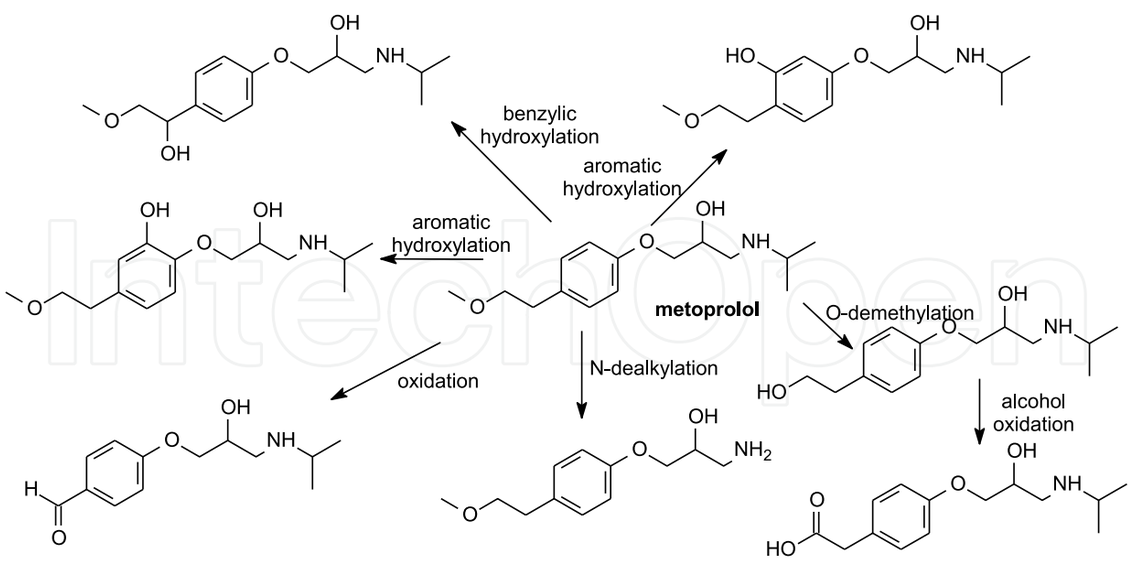


Fig. 5. Metabolism of metoprolol in HLM (Johansson et al., 2007).

The hydroxylation of estrogens has also been investigated in an EC system. Investigations with estradiol, which was subjected to EC oxidation, were interesting and produced two hydroxylated metabolites, 2-hydroxyestradiol and 4-hydroxyestradiol (figure 6) (Gamache

et al., 2003). It has been shown that 2-hydroxylation of estradiol to its catechol derivative is a major metabolic pathway in rodent and human livers, whereas 4-hydroxylation to a different catechol represents a minor pathway in the liver. It has also been demonstrated that 2-hydroxylation is the major hydroxylation pathway in HLM, while the rate of 4-hydroxylation is 1/4 to 1/6 of that for the 2-hydroxylation of estradiol (Zhu & Lee, 2005).

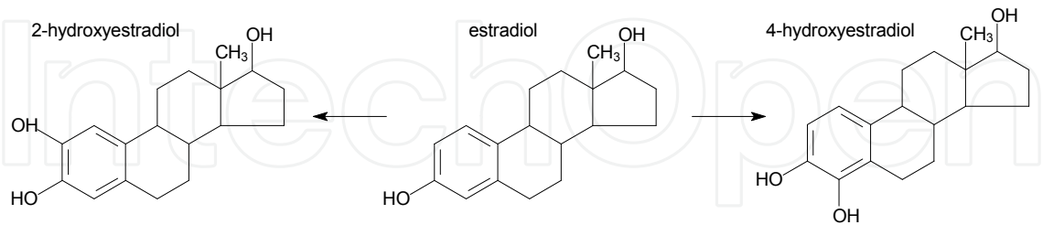
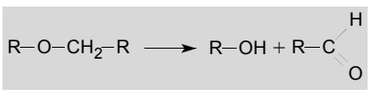


Fig. 6. Hydroxylation of estradiol produced by the EC system at 900 mV as well as in RLM and HLM.

N-, S- and O-Dealkylations



The dealkylation of amines and ethers can be replicated by EC systems. While the former can be readily mimicked, the dealkylation of ethers can only be obtained in low yields. However, the dealkylation of thioethers cannot yet be mimicked using EC.

The dealkylation of alkylamines is the main pathway in both EC and enzymatic oxidation. The parent compound undergoes one-electron oxidation to yield a heteroatom-centered cation radical. The cation radical is better stabilized by sulfur and phosphorus than by nitrogen. As a consequence, the main products from CYP-catalyzed oxidation and from EC oxidation are sulfoxides and phosphine oxides. If the heteroatom is oxygen, CYP is not able to complete a one-electron oxidation due to the poor ability of oxygen to carry the positive charge. Thus, the reaction proceeds via a direct hydrogen abstraction from the α-carbon. In the proposed mechanism for the EC oxidation of aliphatic amines (figure 7), the first two steps are the same for EC and CYP-catalyzed oxidation. One-electron oxidation gives an iminium cation radical, which produces the α-carbon-centered radical. In the CYP-catalyzed mechanism, the electron and the proton are transferred to the iron-oxygen of the enzyme, and the hydroxyl group is introduced to the α-carbon by reacting with the $[Fe–OH]^{3+}$ species. In the EC oxidation, the electron is transferred to the working electrode, and the proton is lost to any compound acting as a base in solution. Because there is no active iron-oxygen intermediary present in the EC system, the neutral radical is further oxidized at the working electrode to the iminium ion. Finally, hydrolysis of the iminium ion provides the dealkylated amine and the corresponding aldehyde (Jurva et al., 2003).

Many drugs contain substituted amine groups. For example, metoprolol undergoes N-dealkylation of the secondary amine and generates a product corresponding to a loss of its isopropyl group. This has been observed in both RLM and HLM, and the reaction has been successfully mimicked using conventional EC, EC-Fenton and porphine systems (figure 5). However, N-deethylation of the tertiary amine function of lidocaine has been observed in

HLM, and the same N-dealkylation was demonstrated for amodiaquine in RLM and HLM. Such N-dealkylation reactions were successfully mimicked in the EC system for lidocaine, while for amodiaquine, the reaction was mimicked in the EC and EC-Fenton systems (Johansson et al., 2009; Lohmann & Karst, 2007).

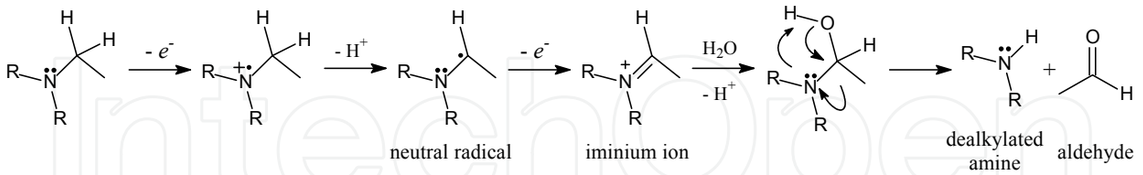


Fig. 7. Electrochemical oxidation mechanism of aliphatic amines.

More recently, an on-chip electrochemical cell was developed for routine use in drug metabolism studies. The chip was used for the metabolism study of the known antiarrhythmic drug procainamide, which found that the chip was able to generate all of the known metabolites produced in both RLM and HLM (figure 8). In the RLM incubation mixture, the metabolites N-hydroxylamine and N-oxide derivatives were identified. In contrast, in the HLM incubation mixture, the dealkylation product N-deethyl procainamide and the oxygenation product N-hydroxylamine derivative were detected. Using an on-line system consisting of the on-chip electrochemical cell, LC separation and subsequent MS detection, the metabolism of procainamide was achieved. In this case, on-chip oxidation actually demonstrated the ability to provide more information about the generated metabolites in a single experiment than the microsomal studies (Odijk et al., 2010).

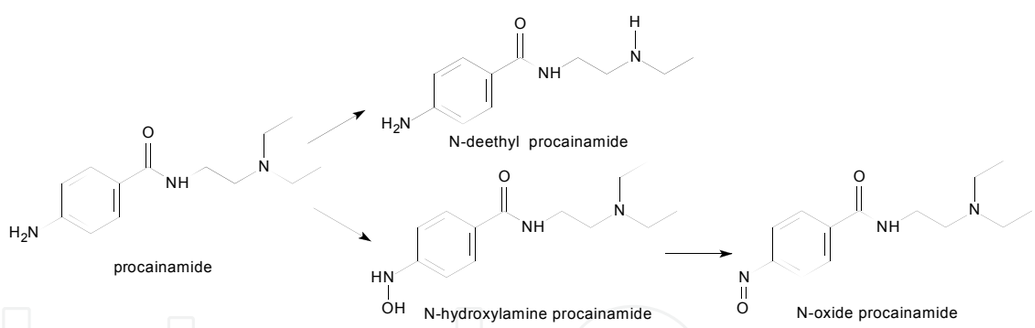


Fig. 8. Oxidative metabolism of procainamide (adapted from Odijk et al., 2010).

Finally, examples of O-dealkylations obtained from EC oxidation include metoprolol, which undergoes O-demethylation (figure 5), and 7-ethoxycoumarin, which generates its corresponding O-deethyl derivative. For both drugs, the same reactions have been found in RLM and HML (Johansson et al., 2007).

Heteroatom (N, S, P) oxidation

As a general rule, S-oxide and N-oxide formation can be successfully mimicked using EC, but unlike the formation of S-oxides, N-oxide formation can only be detected in low yields.

Thus, heteroatom (N, S and P) oxidation has successfully been proven for different drugs when compared with the typical *in vitro* systems. In all cases, the three types of oxidation were observed in both RLM and HLM, and the oxidation was mimicked in the EC, EC-Fenton and porphine systems. Typical examples of such reactions include lidocaine (N-oxidation) and S-methylthiopurine (S-oxidation). In figure 9, representative reactions of each type are presented. In heteroatom oxidation, the porphine system and the EC-Fenton system are preferable due to higher yields of the oxidation product than the EC-system (Jurva et al., 2003).

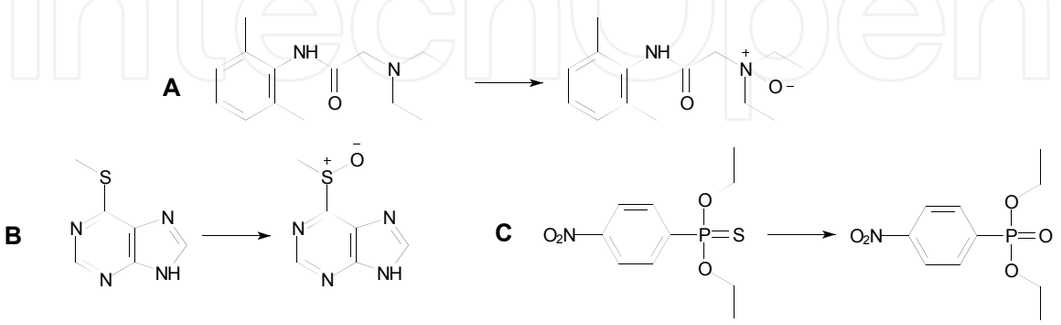
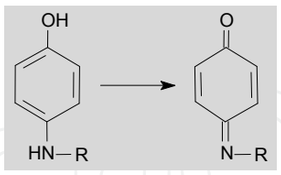


Fig. 9. Typical examples of heteroatom oxidations. A) lidocaine (N-oxidation), B) S-methylthiopurine (S-oxidation) and C) parathion (P-oxidation).

Compounds containing phosphorus generally have a lower oxidation potential than sulfur-containing compounds, and CYP-catalyzed oxidation and electrochemical oxidation of phosphorus-containing compounds almost exclusively result in phosphine oxides. An example of this kind of reaction is the CYP-catalyzed phosphothionate oxidation of the pesticide parathion (figure 9). Indeed, electrochemical oxidation of parathion yielded the same oxidation product at a potential of 600 mV in 0.1 M acetic acid (Jurva et al., 2003).

Dehydrogenation



Dehydrogenation reactions can be readily mimicked using EC, especially when followed by quinone or quinone imine formation. Acetaminophen is oxidized by several human CYP enzymes to its toxic metabolite N-acetyl-p-benzoquinoneimine. The dehydrogenation of acetaminophen has been mimicked by the EC system as well. The proposed mechanism for its EC oxidation included the formation of a radical intermediary, likely via electron transfer followed by proton abstraction. Then, another one-electron oxidation followed by a second proton abstraction yields the final product. This mechanism for EC oxidation is nearly the same as the suggested enzymatic mechanism (Jurva et al., 2003).

A similar reaction occurs with the oxidation of the aminophenol moiety of the antimalarial agent amodiaquine to form the quinoneimine derivative (shown in figure 10). This has been observed in both RLM and HLM, and mimicry has been achieved with the EC-system, the

EC-Fenton system and the porphine system. The generated quinoneimine is an electrophilic species that reacts with nucleophiles present in the system. Therefore, cysteine is usually added to all of the systems, and the detection of amodiaquine cysteinyl conjugates is evidence of quinoneimine formation (Johansson et al., 2007; Lohmann & Karst, 2007).

Other oxidation reactions

Alcohol and aldehyde oxidation. Metoprolol has also been used to illustrate alcohol and aldehyde oxidation. Presumably, the formation of the final carboxylic metabolites from the O-demethylation of metoprolol, shown in figure 5, includes alcohol oxidation to the corresponding aldehyde, which is followed by aldehyde oxidation to carboxylic acid. Both alcohol and carboxylic derivatives have been found in RLM and HLM. These derivatives were mimicked in the porphine system, but only the O-demethylated derivative has been generated in the EC and EC-Fenton systems. The aldehyde derivative that comes from the oxidation of the side chain of metoprolol (figure 5) has been successfully obtained using EC, EC-Fenton and porphine systems but has only been observed in RLM (Baumann & Karst 2010; Jurva et al., 2003).

Benzylic hydroxylation. This kind of oxidative reaction has been mimicked using EC, EC-Fenton and the porphine systems for metoprolol. This metabolite (in figure 5), which is formed through benzylic hydroxylation, is the major hydroxylated metabolite of metoprolol in both RLM and HLM (Baumann & Karst 2010; Jurva et al., 2003).

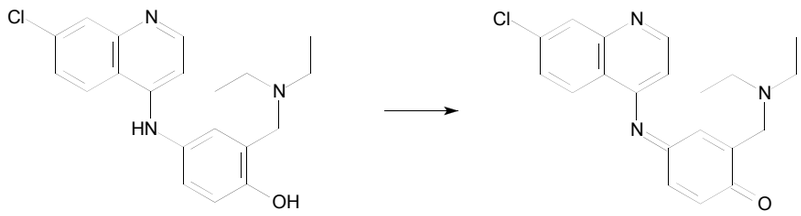


Fig. 10. Formation of the quinoneimine derivative from amodiaquine.

4. Interaction studies

Drug metabolism is regarded as a process that contributes to detoxification, but in some cases, the produced metabolites are reactive species that are more toxic than the parent drug. These reactive intermediates, which are classified as electrophiles and free radicals, can covalently bond to certain endogenous molecules, resulting in cell damage and toxicity.

The application of cyclic voltammetry can provide a first approach to study the reactivities of electrochemically generated species toward endogenous molecules, such as GSH, proteins and DNA. Usually, incubation with biomolecules in an electrochemical cell produces the appearance or disappearance of electrochemical signals in typical voltammograms of the drug of interest. These changes are due to adduct formations between the reactive species and the biomolecules, which could be part of the biological detoxification process. Furthermore, this technique allows an estimate of the electrochemical potential necessary to generate a desired product from the oxidation or reduction of its parent compound. Nevertheless, the small amount of electrolyzed product obtained from this technique does not allow the identification of the adducts formed. For this reason, the controlled potential

electrolysis technique is useful for generating considerable amounts of reactive intermediaries, thereby facilitating the identification of adducts formed using other techniques, e.g., MS.

In this sense, EC-MS and EC-LC-MS systems allow the electrochemical generation and direct detection of reactive intermediaries that are short-lived and unstable, which are difficult to detect using the conventional methods of studying drug metabolism. The incorporation of biomolecules into the electrochemical cell or between EC and MS, as is presented in figure 11, is useful for determining the reactivity of metabolites and the respective adducts formed. This system has already been applied to study the interactions between endogenous molecules, especially with GSH, making it possible to establish relationships and obtain more information about phase II metabolism and drug detoxification mechanisms.

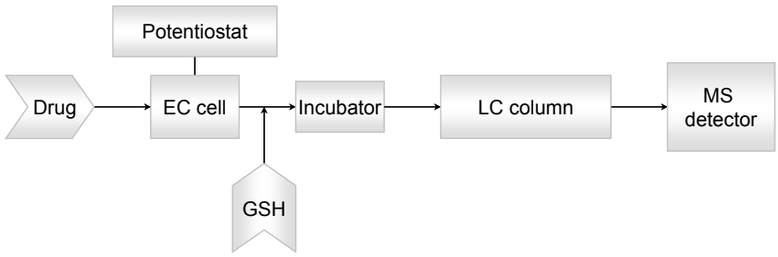


Fig. 11. Schematic representation of the on-line EC-LC-MS system to study adducts formation with GSH or other biomolecules (adapted from Baumann & Karst, 2010).

By using an on-line system, it has been possible to mimic metabolic reactions that involve phase I oxidation products and phase II conjugates of different drugs, such as clozapine, acetaminophen, toremifene, amodiaquine and mitoxantrone, among others (table 1). Some examples of interaction studies and the importance of the information obtained are described below.

The antipsychotic drug clozapine undergoes extensive hepatic metabolism, with the major metabolites found in humans being clozapine N-oxide and N-demethylclozapine. In addition, many other metabolites and adducts have been identified in humans and rats.

Cyclic voltammograms of clozapine have shown one signal in the oxidative scan direction (O1 at 450 mV) and two signals in the reverse scan (R1 at 390 mV and R2 at 75 mV) (figure 12). The potential difference between the O1 and R1 signals revealed a reversible redox process, which means that the oxidation product of clozapine (nitrenium ion) was reduced to the parent molecule. The second signal, R2, was attributed to a chemical reaction subsequent to the clozapine oxidation, with the formation of a new reduced species (hydroxylated). Upon repetitive scans, a new oxidation peak appeared (O2 at 300 mV), which was probably related to the oxidative process of the R2 product (quinone imine derivative). In the presence of GSH, the reduction peaks R1 and R2 disappeared completely in the presence of a ten-fold excess of GSH. These changes in the cyclic voltammetric profiles were due to adduct formations between GSH and the generated reactive intermediaries. Interestingly, upon increasing the GSH concentration, two new oxidation peaks appeared at potentials more positive than O1, which indicated that oxidation of the produced thioadducts occurred.

Using EC-MS and EC-LC-MS systems with a working electrode consisting of porous glassy carbon, different metabolites were obtained for clozapine, depending on the applied potential (figure 13). Upon oxidation to 400 mV, hydroxyl clozapine derivatives and nitrenium ions were the predominant products formed. Increasing the potential to 700 mV, the generation of an additional product, N-demethyl clozapine, was observed. No N-oxide derivative was detected by this system, probably because it was only generated to a small extent. When the oxidation of clozapine was performed in the presence of GSH, the formation of adducts between the metabolites that were generated and GSH were detected. These adducts were identified as hydroxy-GSH clozapine and GSH-demethyl clozapine. Additionally, a bis-GSH adduct and at least three isomers of a GSH-clozapine adduct were formed. Despite the fact that not all oxidative metabolic pathways of clozapine were mimicked by this system, the production of similar metabolites (phases I and II) confirmed the *in vitro* and *in vivo* reports concerning the metabolism of clozapine (van Leeuwen et al., 2005).

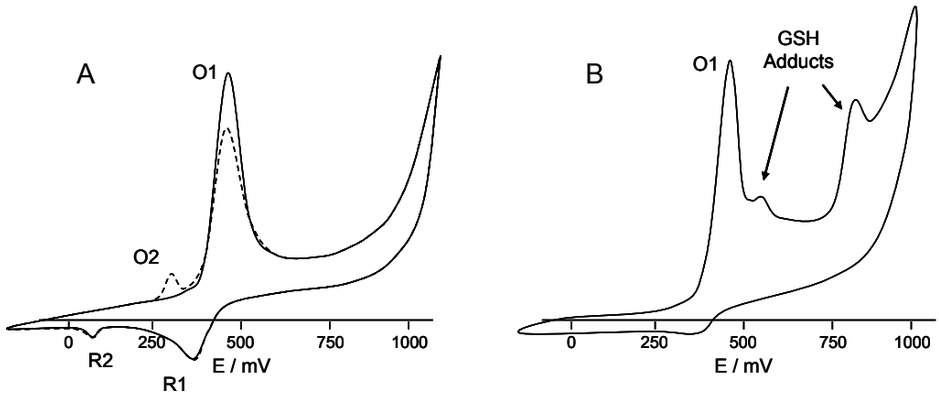


Fig. 12. Cyclic voltammograms of clozapine (A) without GSH and (B) with GSH (adapted from van Leeuwen et al., 2005).

The most studied drug in metabolite-biomolecule interactions is acetaminophen, and it is a classic example of the importance of these studies to measure the potential metabolic toxicity of a substance. Acetaminophen is a drug that is well known to cause liver toxicity in humans by its oxidative metabolic pathway in which the highly reactive electrophile intermediate N-acetyl-*p*-benzoquinoneimine (NAPQI) is generated. This species is normally detoxified by conjugation with reduced GSH, but in an acetaminophen overdose, the total liver GSH is depleted. EC-MS and EC-LC-MS experiments using either acetaminophen alone or in the presence of GSH or N-acetylcysteine (NAC) have been conducted to study the detoxification pathway of acetaminophen.

The electrochemical oxidation of acetaminophen leads to NAPQI, which is also observed *in vivo*. After applying a potential of 600 mV, at which acetaminophen shows an oxidation wave in cyclic voltammetry measurements, three major additional signals were observed in the presence of GSH or NAC, which indicated the formation of acetaminophen adducts. In the presence of these thiol-containing molecules, NAPQI was quenched by adduct formation (figure 14). This mechanism is the common detoxification pathway of NAPQI in the human liver. MS measurements showed only the formation of GSH and NAC monoadducts, but in each case, two different isomers were produced, which could

correspond to 2- and 3-conjugated-acetaminophen. In general, NAPQI has two possible reaction sites, namely, the 2- and 3-substitution positions. Unlike in the human body, only conjugates at position 2 of acetaminophen are formed. This selectivity is a result of the CYP enzymatic system that is involved in *in vivo* adduct formation, which is not possible to achieve using only electrochemical methods and trapping agents. Despite this, the electrochemical oxidation of acetaminophen, alone and in the presence of GSH or NAC, allowed the successful replication of the metabolic detoxification pathway that occurs in the human liver (Lohmann et al., 2006).

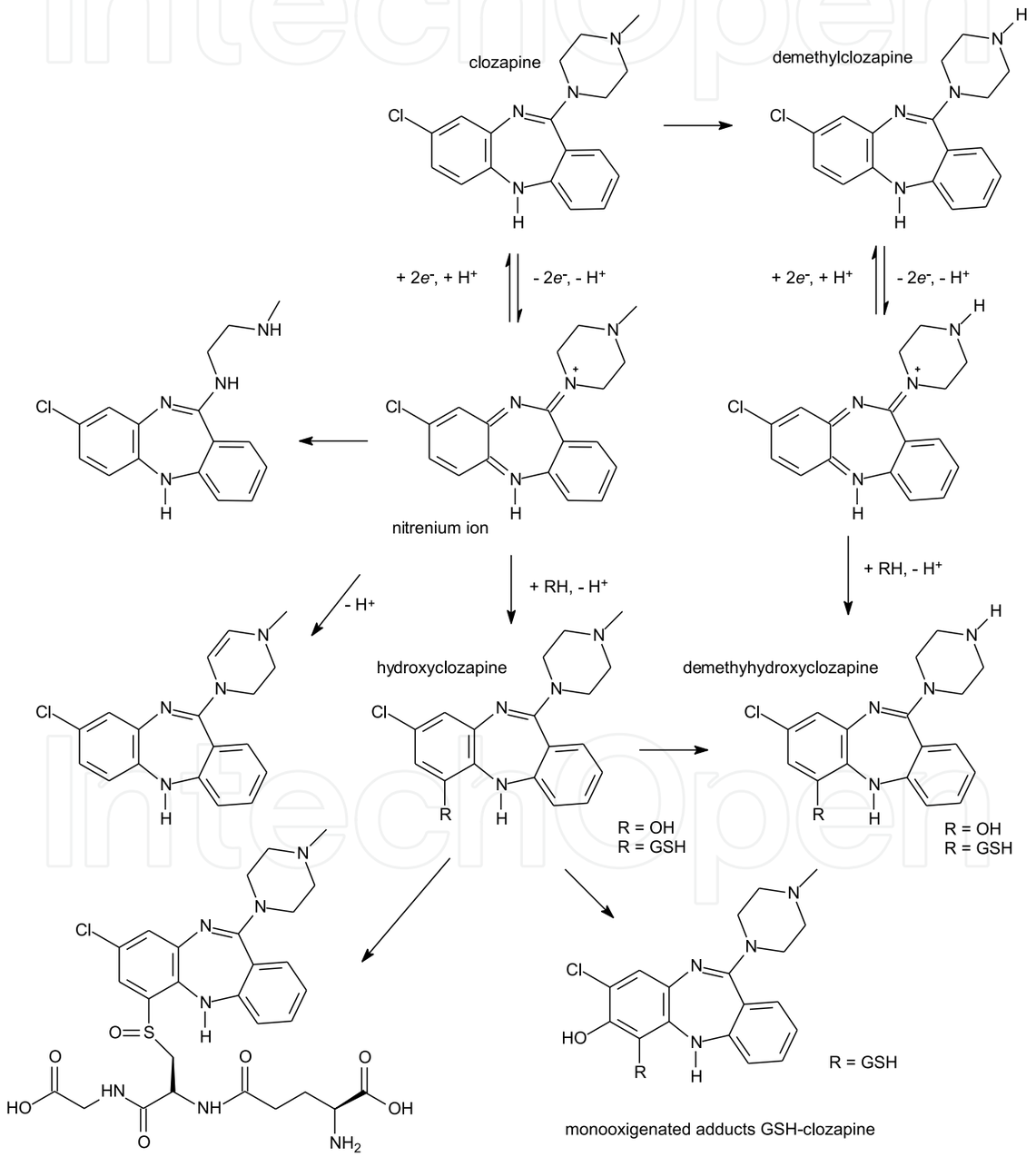


Fig. 13. Electrochemical oxidation of clozapine (van Leeuwen et al., 2005).

A modification of the conventional on-line system was introduced to simulate conjugative phase II reactions. This system consisted of the incorporation of an enzymatic reactor between the electrochemical cell and the separation system and was applied to investigate the phase II metabolism of toremifene, a selective estrogen receptor modulator.

It is well known that toremifene metabolism by CYP is comprised mainly of hydroxylation, O-dealkylation, N-dealkylation and N-oxide formation, which are observed in rat, mouse, and human liver microsomes. All are produced electrochemically, except for the N-oxide derivative (figure 15). Furthermore, the generation of reactive quinone methides may occur, which are detoxified by reacting with GSH, the reaction of which is catalyzed by glutathione-S-transferase (GST).

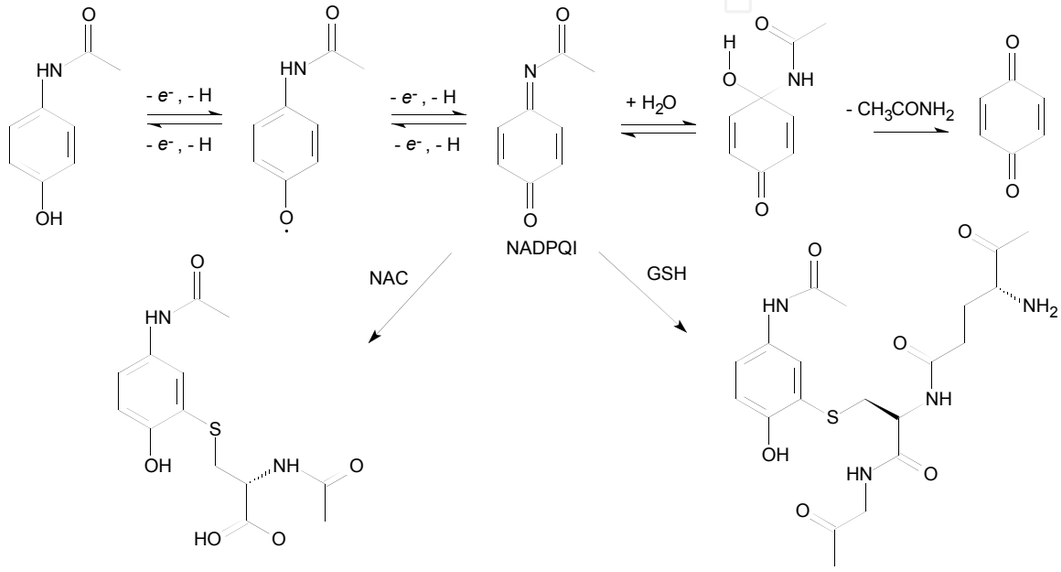


Fig. 14. Detoxification mechanism of acetaminophen by adduct formation with GSH and NAC (Lohmann et al., 2006).

Generally, the conjugation of quinoid compounds with GSH observed in phase II metabolism has been successfully mimicked by electrochemical methods because the conjugation proceeds spontaneously via a 1,4-Michael addition reaction. In the case of toremifene, the conjugation with GSH occurs by nucleophilic substitution of the chloride atom, which normally is not possible using electrochemical techniques, which suggests that this reaction requires enzymatic catalysis to proceed. However, using only an EC-LC-MS system with GSH incubation, it was possible to identify the adduct formed between GSH and the quinone methide derivative by chloride atom substitution. Furthermore, the incorporation of GST to the system made it possible to obtain an additional conjugate that is only generated by enzymatic catalysis. Thus, the use of metabolic enzymes in the on-line EC-LC-MS provided important information about metabolites that are not normally identified by conventional *in vitro* and *in vivo* assays or by simple electrochemical systems (Lohmann & Karst, 2009).

Other interesting approaches that involved this on-line system included interaction studies of metabolites with more complex biomolecules, such as plasma proteins and DNA. To

perform and evaluate the covalent protein binding of reactive phase I metabolites, the oxidation products of acetaminophen, amodiaquine and clozapine have been evaluated with regard to adduct formation with beta-lactoglobulin A and human serum albumin. The experiments demonstrated that mainly covalent binding took place between the free thiol groups of these proteins and the reactive metabolites, which is analogous to endogenous detoxification pathways. Thus, this experimental system offered interesting features in the risk assessment of the covalent protein binding of drug metabolites due to the significance of the protein structure and its function (Lohmann et al., 2008).

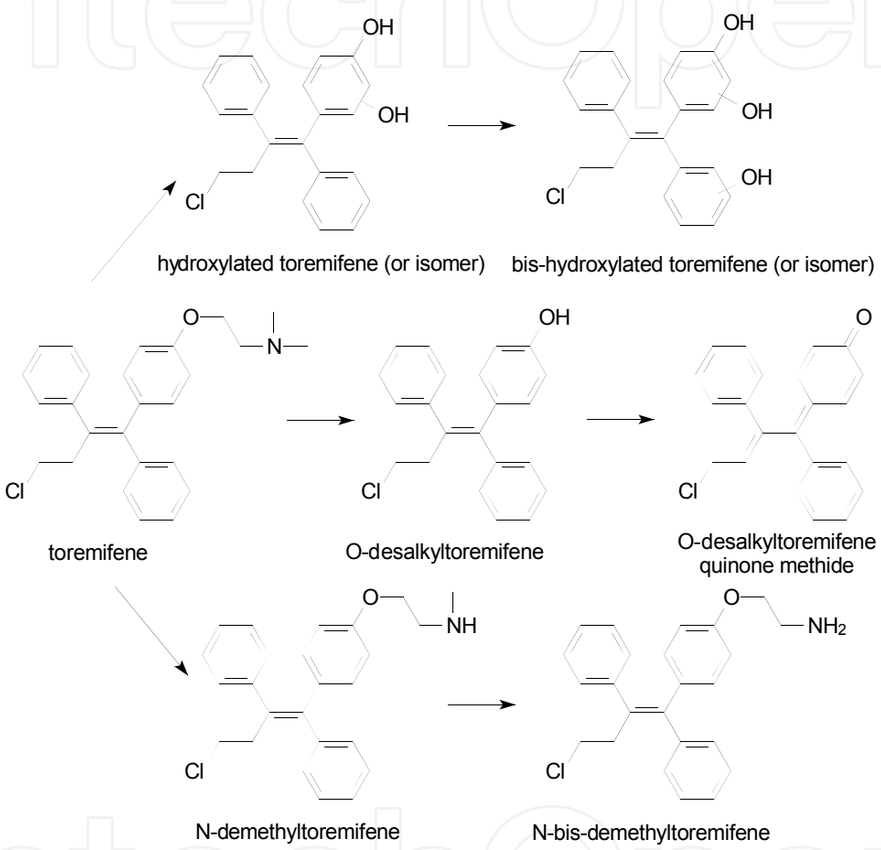


Fig. 15. Electrochemical oxidation of toremifene (Lohmann & Karst, 2009).

In relation to reductive metabolism, the redox chemistry of different nitro compounds of biological significance have been studied to understand how the reduction of the nitro group can play an active role in free radical generation and free radical reactivity. Some drugs that were studied corresponded to calcium antagonists, antibacterial and antiprotozoan agents, among others (Squella et al., 2005). Cyclic voltammetric technique using mercury as the working electrode permitted the simulation of the enzymatic formation of the nitro radical anion, a product of the one-electron reduction of nitro compounds, and an evaluation of chemical and biological characteristics.

Using this electrochemical technique, it was also possible to detect and quantify the interaction between the nitro radical anion derivative and several targets of biological significance, such as oxygen, thiols and DNA. In fact, the addition of a target compound

with which the nitro radical anion derivative could interact produced changes in the cyclic voltammetric response that could be used to quantitatively calculate the interaction constant value. One of the main advantages of cyclic voltammetry in the study of nitro radical anions is that they can be generated and studied *in situ* (Bollo et al., 2000).

5. Biosensors for metabolic reactions

A biosensor is a device capable of detecting, recording and transmitting information about the presence, activity or concentration of a specific compound in solution with a biological recognition element and a physicochemical transducer (figure 16). These devices have become very important due to the advantages they possess as rapid tools for the screening and detection of substances and the possibilities of miniaturization that would allow the development of portable devices.

As can be seen in figure 16, the biological recognition element can include enzymes, antibodies, cell receptors, tissues, or microorganisms, whereas the transducers that are often used are electrochemical, optical and piezoelectrical. The biorecognition process can either be a bio-affinity (affinity ligand-based biosensor) or bio-metabolism (enzyme-based biosensor) reaction, and this interaction results in biochemical and physicochemical changes that are converted to a quantifiable signal by the transducer. The biosensor selectivity for the target drug is mainly determined by the recognition element, while its sensitivity is greatly influenced by the transducer. In this sense, the most common biosensors are based on electrochemical transducers that confer high detection sensitivity (D'Orazio, 2003). In electrochemical biosensors, the main transducing elements include working electrodes of noble metals (e.g., platinum or gold) or carbon derivatives (e.g., glassy carbon or carbon paste). These electrodes can be modified to improve the connection to the recognizing agent, which would make them applicable to a large variety of samples.

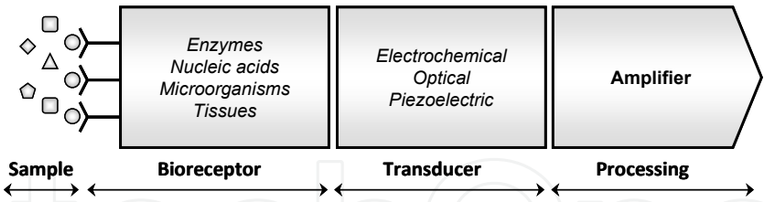


Fig. 16. Representation of a typical biosensor, including the different constituent parts.

Although biosensors have been successfully developed and used in different areas, with the glucose biosensor being the most common example, their application in metabolism is relatively new, and most of the works in this area have focused on optimizing the immobilization process of the biological element. Because most electrochemical biosensors are based on enzymatic reactions, they are a promising strategy for studying metabolic reactions, particularly by CYP-catalyzed oxidations. For example, modified electrodes with CYP enzyme films could be used as a screening tool to study drug metabolism, focusing mainly on drug interactions with the enzyme and on the toxicity of metabolites toward biomolecules, such as proteins and DNA (Bistolas et al., 2005).

A critical step in the construction of any biosensor is the immobilization of the biorecognition element onto the electroactive surface because an incorrect immobilization can lead to the loss

of the native structure and therefore a loss of activity of the biological compound. In general, direct electrochemistry of CYP enzymes on unmodified electrodes has proven very difficult due to the deeply buried heme cofactor and instability of the biological matrix upon interaction with the electrode surface. To solve this problem, an immobilization material can be used, which is often a membrane or matrix that acts only as a biological component support or that participates in the signal transduction system. In addition to this problem, when the CYP is used as the biorecognition element, an extra component to provide external electrons (e.g., flavin, iron–sulfur proteins or NAD(P)H) is required, which makes biosensor development more complex. Despite these limitations, there are results that suggest that human enzyme CYP3A4 immobilized onto a gold electrode is electrically and catalytically active (Joseph et al., 2003). Additionally, some authors have studied the possibility of a direct electrochemical delivery of the required electrons using the CYP catalytic cycle, which would result in an interesting alternative that facilitates biosensor development without the need of redox transfer proteins and cofactors (Bistolas et al., 2004; Yang et al, 2009).

Yang et al. developed an electrochemical biosensor for the mimicry metabolism of warfarin, which is almost exclusively biotransformed by CYP2C9 to the primary inactive metabolite 7-hydroxy-warfarin and to 6-hydroxy-warfarin. The system consisted of the immobilization of CYP2C9 onto a gold electrode through an 11-mercaptoundecanoic acid and octanethiol self-assembled monolayer, without the presence of cofactors or other redox transfer proteins (figure 17). Using cyclic voltammetry, the reduction peak on the forward scan suggested the direct electron transfer from the electrode to the heme group of the enzyme and the consequent oxidation of the substrate, whereas the peak on the reverse scan indicated the re-oxidation of the reduced enzyme. The electrochemical behavior of this system demonstrated that warfarin was oxidized by CYP2C9. By controlled potential electrolysis and analysis of the electrolyzed solution using LC, the metabolite 7-hydroxywarfarin was identified, which was also obtained in an *in vitro* enzymatic assay (Yang et al, 2009). Thus, the developed system was efficient in the generation of the main metabolite of warfarin and therefore could be used to mimic oxidative drug metabolism in a more accurate form, without the necessity of enzymatic cofactors.

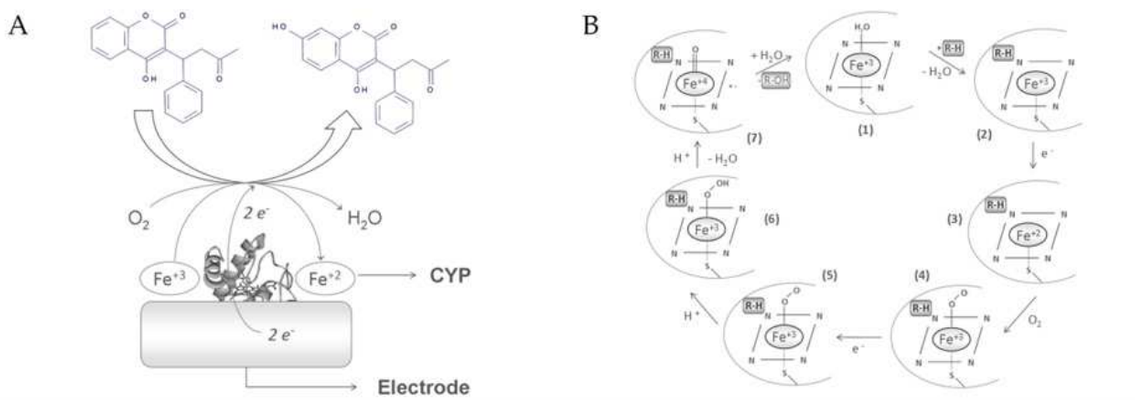


Fig. 17. A) Schematic representation of the CYP2C9 biosensor and the electrochemical oxidation of warfarin catalyzed enzymatically (adapted from Bistolas et al., 2005). B) Catalytic cycle of the CYP heme group and the reactions of the oxidation process (adapted from Lohmann & Karst, 2008).

A similar metabolic biosensor using the more predominant isoform of cytochrome P450 CYP3A4 was developed based on the redox properties and electronic transfer of the heme protein. In this case, the alternate adsorption of a CYP3A4 layer on top of a polycation layer was assembled onto gold electrodes. Under anaerobic conditions, a reversible electron transfer between the electrode and CYP3A4 was observed using cyclic voltammetry. However, in the presence of oxygen, the reduction peak increased 2- to 3-fold, and the oxidation peak of the hemoprotein disappeared. The addition of CYP3A4 substrates, such as verapamil, midazolam, quinidine and progesterone, to the oxygenated solution caused a concentration-dependent increase in the reduction current in cyclic voltammetric and amperometric experiments, which indicated an interaction between the enzyme and substrate. Controlled potential electrolysis of verapamil and midazolam and analysis by LC-MS revealed that the metabolites generated by this system were comparable to those produced by the microsomal incubation of human CYP3A4 with an NADPH-generating system. In addition, the presence of ketoconazole or catalase in the medium inhibited the catalytic activity of the enzyme and consequently led to a reduction in the amount of metabolites generated. Thus, important information was obtained from this system in the evaluation of new drugs as potential substrates or inhibitors of CYP3A4 (Joseph et al., 2003).

Inhibition and induction studies of metabolic enzymes are important in drug development processes because they can modulate or change the toxicity related to a drug. In this sense, Hull et al. developed an electrochemical biosensor to screen the metabolic inhibition of xenobiotics. This device consisted of films of bacterial cytochrome P450cam and DNA on a pyrolytic graphite electrode using a $Ru(bpy)_3^{2+}$ complex as a mediator of DNA voltammetric oxidation. For this study, styrene was chosen as the enzymatic substrate because its metabolite is genotoxic toward guanine in DNA. Styrene metabolism initiated by hydrogen peroxide was evaluated with and without the following inhibitors: imidazole, imidazole-4-acetic acid and sulconazole. The initial rates of DNA damage decreased with increasing inhibitor concentrations, and the calculated inhibition constants were directly related to the changes in styrene metabolism and DNA adduct formation in the presence of the inhibitor, which confirmed the usefulness of this enzyme/DNA biosensor as a rapid screening tool for metabolic inhibition studies of pharmaceutical candidates (Hull et al., 2009).

Therefore, modified electrodes with metabolic enzymes could provide significant information about metabolism, drug-drug metabolic interactions and whether a drug is the substrate of a specific enzymatic isoform. Furthermore, the possibility of incorporating different biomolecules into this system may provide additional advantages to the study of drug toxicity. In this sense, DNA biosensors that have been developed could be used in the future to assess the genotoxicity of drug metabolites.

6. Conclusions

The use of electrochemistry, a purely instrumental technique, has emerged as an interesting alternative to generate and detect metabolites in drug development processes because it can mimic the main oxidative reactions that occur in the human body, providing a good approximation of what occurs *in vivo*. However, it is important to take into account that not all compounds can be electrochemically oxidized; therefore, a complementary approach to this system is necessary, such as the incorporation of enzymes useful for studying metabolism.

Electrochemistry exhibits certain advantages compared to conventional methods because it allows the generation and direct identification, in a rapid and clean manner, of both stable species and metabolites with short half-lives without the need to use laboratory animals or organ extracts. Additionally, it can be a powerful tool in the interaction studies with specific cellular components, which is more difficult to realize with *in vivo* or *in vitro* methods.

In the future, this technique will most likely be supplemented with different enzymes or cofactors to expand its coverage to all metabolic reactions. It appears that biosensors could follow that trajectory to an increase in coverage and specificity. Additionally, the development of biosensors based on the use of metabolic enzymes and a biomolecular target that is susceptible to damage by the generated metabolites is a promising strategy to investigate enzyme inhibition in future metabolism studies.

7. References

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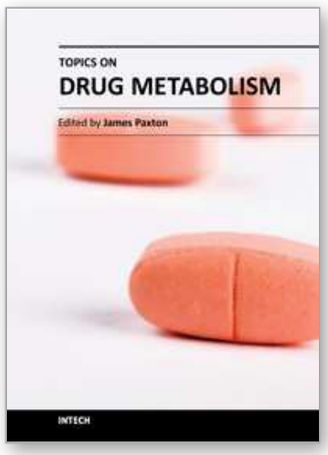

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In order to avoid late-stage drug failure due to factors such as undesirable metabolic instability, toxic metabolites, drug-drug interactions, and polymorphic metabolism, an enormous amount of effort has been expended by both the pharmaceutical industry and academia towards developing more powerful techniques and screening assays to identify the metabolic profiles and enzymes involved in drug metabolism. This book presents some in-depth reviews of selected topics in drug metabolism. Among the key topics covered are: the interplay between drug transport and metabolism in oral bioavailability; the influence of genetic and epigenetic factors on drug metabolism; impact of disease on transport and metabolism; and the use of novel microdosing techniques and novel LC/MS and genomic technologies to predict the metabolic parameters and profiles of potential new drug candidates.

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