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Disclosing the redox metabolism of drugs: the essential role of electrochemistry.

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Abstract

Electrochemical methods provide a wide range of strategies to explore the metabolism of drugs. These approaches traditionally encompass preparative aspects viz. the electrochemical generation of potent metabolites or the electrochemical exploration of the reactivity of redox enzymes (or their mimics) towards drugs. More recently the electroanalytical characterization of the successive redox and redox-coupled reactions was found effective to unravel more complex mechanisms, especially those related to the reactivity of bioorganometallic drugs. This minireview highlights the contribution of these different electrochemical strategies to the determination of drug metabolism through representative recent examples.

Keywords

Molecular electrochemistry, Metabolism, Mechanism, Drugs

1. Introduction

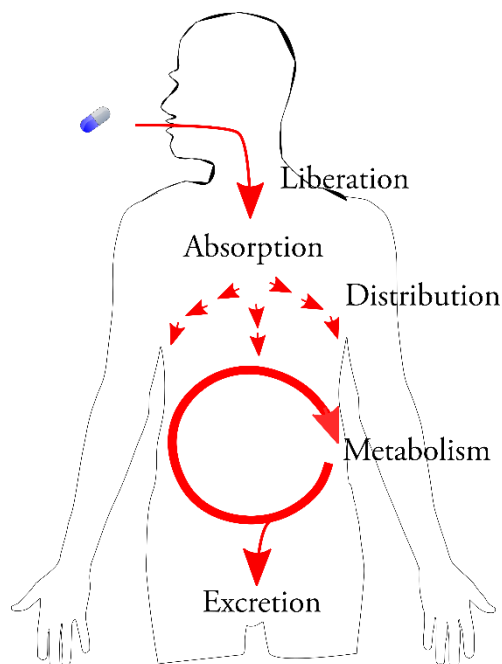
The fate of a drug in a living organism is often depicted in pharmacokinetics by the ADME acronym[1] or more accurately LADME –Liberation, Absorption, Distribution, Metabolism, Excretion- [2] (Scheme 1). In that sequence, chemical transformations mostly relate to the metabolic part. Specifically, the *in vivo* transformation of a molecule meets a large number of redox steps, a representative example being those catalyzed by cytochrome(s) P450, i.e. iron hemoproteins acting as oxidases/monooxygenases[3, 4].

While the conversion of simple substrates (O₂, NO, glucose,...) produces unambiguous metabolites, the pathways followed by molecules of higher structural complexity are far more difficult to decipher since the number of chemical steps required for their metabolism increases accordingly. As a consequence, the identification of a specific intermediate as being the actual biologically-active species in a multistep metabolism remains challenging. Moreover, the advent of bioorganometallic drugs in the past two decades[5, 6] increased the quest for coherent frameworks on the successive chemical steps involving these compounds in which the oxidation state of the metal centers strongly influences the reactivity of the organic functions.

In that context, the remarkable capacity of electroanalytical methods to provide both thermodynamic and kinetic information not only on a given redox species, but also on the preceding or consecutive chemical steps [7] makes electrochemistry an approach to be privileged in the exploration of complex metabolisms. After a short survey of the “traditional” use of electrochemistry in pharmacokinetics in

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the first two paragraphs, the third paragraph will focus on the recent advances made in the electroanalytical determination of drug metabolism.



Scheme 1. Illustration of the LADME acronym used in pharmacokinetics to describe the Administration, Distribution, Metabolism, and Excretion processes.

2. Electrochemical approaches devoted to the determination of metabolic pathways

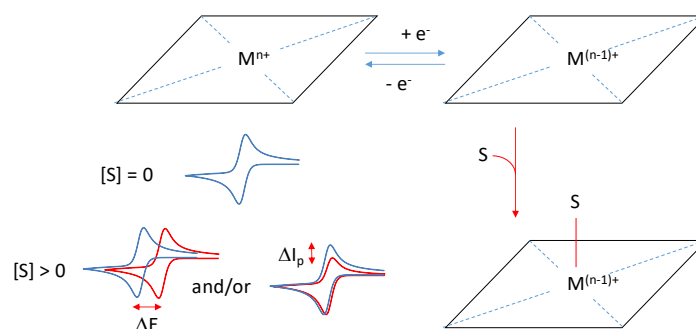
2.1 Electrochemical cells as mimics of biological reactors

If one attempts to make a bibliographic statement on the “electrochemical” determination of drug metabolisms, all databases will at first return a long list of articles dealing with the use of electrochemical cells to mimic the oxidizing conditions met in tissues like liver, or as complementary/alternative assays to microsomal incubation[8-10]. Generally, electrochemical setups are coupled to mass spectrometry detection (hyphenated electrochemistry/MS), in order to identify the stable electrogenerated metabolites. This methodology, where electrochemistry is “preparative” - the term *reactor* is often used – has been downscaled to microfluidics[11, 12] using flow cells, a strategy adapted for the fast generation and collection of a high number of species. Under these conditions, electrochemical oxidation processes (reductive metabolisms are scarcely described using this approach) yield a distribution of metabolites. Then, a reactive framework can be proposed to connect the species detected. However, only stable metabolites can be formed under such statistical generation mode. Transient intermediates, especially with short lifetimes, may probably not be detected and key-species could be discarded if unstable and therefore not listed among the electrolysis products. Besides, the lack of control on the thermodynamics (oxidation potential) and the absence of reliable information on the chemical reactions coupled to the electron transfer makes such electrochemistry/MS protocols inadequate for the determination of step-by-step transformations, a key-issue in the establishment of metabolic pathways. Nevertheless, this approach still prevails in pre-

clinical studies, as evidenced by the large number of recent publications dealing with the metabolism of xenobiotics [13] including pesticides like triclosan [14] as well as antitumor drugs like triapine [15] and acridinone derivatives [16, 17].

2.2 Electrochemical behavior of redox enzymes towards drugs

The reactivity of a biomolecule or a drug is often observed through the activity of an enzyme. Accordingly, redox enzymes are in the spotlight of considerable electrochemical investigations. Indeed, the general metabolic framework of metalloenzymes can be accessed *in vitro* from electrochemical approaches carried out directly on these enzymes or their mimics. The reactivity of a drug or a biomolecule is therefore examined through the modification of the redox properties of the metal center (Scheme 2):



Scheme 2. Characteristic cyclic voltammogram modifications following a reaction between a substrate S (drug) and a metal center (M) mimicking a redox enzyme. The bonding or ligation of the drug (substrate S) to the metal center (square planar representation in this scheme) may result in a potential shift and/or a loss of reversibility of the reduction wave ascribed to the M^n/M^{n-1} process. The effect of time (scan rate) and concentration of S on the amplitude of these modifications allows, under certain conditions, determination of both the thermodynamic and kinetic constants related to the reaction of the M^{n-1} redox state of the enzyme with the substrate S[7].

Although involved in the redox metabolism of a wide range of drugs and xenobiotics[4], cytochromes like P450 have given rise to only few electrochemical characterization[18, 19], probably due to the difficulty in isolating and solubilizing appropriate amounts of this enzyme, which therefore directed electrochemical investigations towards immobilized systems[20-22]. A recent example of P450 related metabolism was explored for omeprazole [23], a proton pump inhibitor used to regulate stomach acidity. Accordingly, iron-centered hydrogenases have been reconstructed at electrodes and their reactivity explored electrochemically [24, 25]. Alternatively, mimicry strategies are developed featuring more simple species possessing the reactive core metal-ligand structure, to address general reactivity points, namely fundamental aspects of proton coupled electron transfers [26-28]. Recent publications also focus on the general reactivity of cytochrome C, a key-electron relaying hemoprotein involved in homeostasis and apoptosis processes [29, 30]. However, such approaches focus on specific steps and remain incomplete to describe full complex metabolic sequences.

2.3 Drug metabolism from a molecular electrochemistry viewpoint

- *Non-organometallic drugs*

Several organic drugs have given rise to electrochemical exploration, either at the level of a specific redox step or considering their evolution under defined oxidizing conditions. This paragraph only reports the investigations devoted to the exploration of metabolisms, not those related to the electrochemical determination of the drug itself, which are connected to strictly analytical purposes. The metabolism of drugs has been addressed by electroanalytical methods as early as 1990 with the study of the reducibility of Mitoxantrone, an antineoplastic agent used to treat e.g. specific leukemia, prostate cancers or multiple sclerosis [31]. The reducibility of this quinone-based drug was established from cyclic voltammograms as the starting point of a reductive metabolic sequence where oxygenated toxic radicals were formed. Another early example was achieved on various anticonvulsants[32], where the reductive behavior of nitrogen compounds namely containing triazole or pyridazine moieties has been studied in order to ensure they could be reduced under biological conditions. More recently, the electrochemical oxidation of Acyclovir, an antiviral agent, has been explored by cyclic voltammetry and the $2e^-/2H^+$ oxidation process observed prompted the authors to propose the formation of an oxo-acyclovir product as early intermediate in its oxidative metabolism[33].

The metabolism of anti-estrogenic molecules like tamoxifen has also been addressed from electrochemical studies with respect to the oxidizing conditions met in cancer cells (*vide infra*). In these studies, the oxidation peak potential dependence of tamoxifen was studied in various pH buffers [34, 35].

A more comprehensive survey of the electrochemically-probed redox reactivity of various quinone-based therapeutic molecules was reviewed by Goulart *et al.*, with a specific emphasis on oxidizing agents related to oxidative stress (ROS/RNS, Reactive Oxygen/Nitrogen Species) together with the inventory of potent targets in the quinone or semi-quinone family [36]. This work addressed quantitative considerations supported by a long-term expertise on the electrochemical monitoring of ROS and RNS at the single cell level, allowing an accurate and realistic mapping of the reactive framework for these compounds.

The interaction of a drug with its targets and potential antagonists in cells can be regarded as the early steps of its metabolism. In this context, electrochemical techniques are particularly adapted to the study of redox-active molecules. Indeed, the effect of antioxidants like caffeic acid was recently monitored electrochemically towards the binding ability of Daunorubicin, a DNA-intercalating molecule used to treat leukemia [37]. A recent example of the interactions between DNA and an antiviral molecule, Tenofovir, was also recently reported [38].

- *Organometallic drugs*

The introduction of metal centers in drugs generally allow a facile characterization of their redox properties through easily switchable redox states. A nice example of organometallic drug is ferroquine, a ferrocene-functionalized antimalarial, which was explored by cyclic voltammetry to characterize both its reducing properties and capacity to promote hydroxyl radical formation from H_2O_2 [39]. Similarly, the reducing properties of ferrocene-functionalized purine derivatives have recently been explored electrochemically in parallel with their antitumoral and ADME features [40]. In fact, ferrocene compounds are in the spotlight of numerous therapeutic and electrochemical evaluations, because of both the accessible ferrocene functionalization chemistry and the their facile electrochemical characterization (fast + reversible oxidation), as recently reported with benzimidazole derivatives [41].

In the last decade, several papers and reviews have emphasized the growing importance of organometallic drugs namely in cancer therapy [42-45]. Actually, oxidative stress, associated to the production of high concentration levels of reactive oxygen species (ROS), is presented as a common feature of a wide range of disorders affecting human health[46]. Under such abnormal oxidative conditions, the metal center of bioorganometallic drugs may undergo oxidation, which often triggers a complex sequence of reactions. The exploration of the successive steps involved upon redox activation of the drug first requires the deployment of quantitative and foremost short-time compatible techniques to intercept the signature of transient intermediates. With this respect, electrochemical methods based on voltammetry and amperometry methods offer a wide time window (from 10 s down to 1 ms – or 10 ns with specific apparatus and expert knowledge)[47]. Furthermore, studying a cascade of reactions requires similar methodologies whatever the scope, biological or chemical. The electrochemical determination of mechanisms encountered in organometallic catalysis has been widely reviewed for metals like palladium[48], cobalt[49] and nickel[50], an inspiring approach to address the redox metabolism of bioorganometallic drugs.

A representative example has been described for ferrocifens, a family of antitumoral compounds displaying a variety of oxidative routes, mostly deciphered thanks to electrochemical approaches [51]. The cytotoxicity of these molecules has originally been associated to their 2-electron oxidative conversion to quinone methides (QM) a common stable metabolite detected both in vivo and in vitro [52]. However, some molecules showed low IC₅₀ (highly toxic) and poor oxidizability, or the opposite, suggesting the occurrence of other biologically-active metabolites than quinone methides. Actually, a thorough voltammetric exploration (see Figure 1 below) allowed the sequential identification of the metabolites involved in the quinone methide formation [53]. A 10 ms-lifetime organic radical, the oxidation and backward reduction of which being only observable at high scan rates (reduction wave Ω in the figure), appeared to act as a key reducing species [54]. Moreover, electrochemical studies have established that analog ruthenociphenol or osmociphenols undergo a different and more complex oxidation sequence, featuring new metabolites that could induce cytotoxicity [55, 56] upon interaction with thioredoxin enzymes.

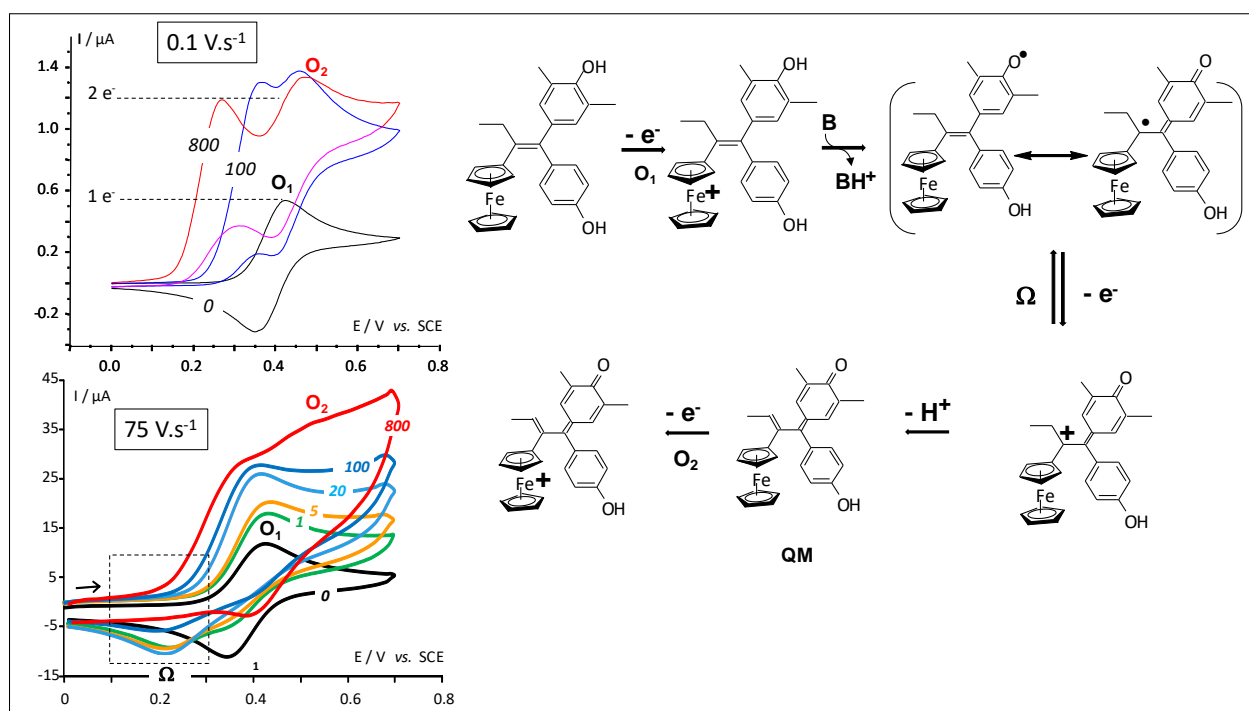


Figure 1. Imidazole concentration-dependent electrochemical behavior of a representative example of hydroxyferrocifen. At $0.1 \text{ V}\cdot\text{s}^{-1}$ and large imidazole excess (800 molar eq. vs the ferrocifen) the $2e^-$ formation of the quinone methide was observed in the negatively shifted red wave. At $75 \text{ V}\cdot\text{s}^{-1}$ a transitory reduction wave Ω appeared at intermediate imidazole excesses, ascribed to the backward reduction of the carbocation at the lower right of the reaction scheme.

In the field of drug metabolism, ferrocifens remain to our knowledge the only examples of a fully characterized oxidation sequence.

3. Concluding remarks

Electrochemistry, which was originally introduced to produce metabolites (mimicking in vivo oxidizing environments) or to characterize the redox states and reactivity of a specific enzyme, has recently gained a considerable visibility in the rigorous stepwise description of metabolic pathways, especially for bioorganometallic drugs. Indeed, the exploration of the reactivity of such compounds can be carried out from strategies and approaches derived from classical organometallic chemistry, thus providing information on short lifetime intermediates as well as a rationale on the thermodynamic and kinetic parameters that may affect specific steps. The overall journey of a drug may therefore be accessible through the establishment of comprehensive, reliable and robust frameworks collected from electrochemical data, a central hinge point in the evaluation of global pharmacokinetic parameters such as the metabolic clearance of a drug [57]. Powerful and reliable, molecular electrochemistry remains a burgeoning approach to explore multistep drug metabolism, and this should incite electrochemists to further immerse in this field where their expertise is unique.

4. References

(*) Papers of special interest

(**) Papers of outstanding interest

[1] T. Hou, X. Xu, ADME evaluation in drug discovery, *Molecular modeling annual* 8(12) (2002) 337-349.

[2] H.J. Olguín, S.R. Pérez Saldaña, I.L. Asseff, Meaning of pharmacokinetic parameters, *Optimization of Drug Prescribing in Elderly*, Nova Science Publishers, Inc.2016, pp. 57-62.

[3] P.B. Danielson, The cytochrome P450 superfamily: Biochemistry, evolution and drug metabolism in humans, *Current Drug Metabolism* 3(6) (2002) 561-597.

[4] U.M. Zanger, M. Schwab, Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation, *Pharmacology & Therapeutics* 138(1) (2013) 103-141.

[5] *Bioorganometallic Chemistry: Applications in Drug Discovery, Biocatalysis, and Imaging*, 1st ed., Wiley-VCH 2015.

[6] C.G. Hartinger, P.J. Dyson, Bioorganometallic chemistry—from teaching paradigms to medicinal applications, *Chemical Society Reviews* 38(2) (2009) 391-401.

(**) [7] J.M. Saveant, C. Costentin, *Elements of Molecular and Biomolecular Electrochemistry: An Electrochemical Approach to Electron Transfer Chemistry*, 2nd ed., John Wiley & Sons, Inc., Hoboken, New Jersey, 2019.

This book recently updated is the most comprehensive manual dealing both with theoretical and practical aspects related to the electrochemical determination of reaction mechanisms

- [8] H. Faber, M. Vogel, U. Karst, Electrochemistry/mass spectrometry as a tool in metabolism studies-A review, *Analytica Chimica Acta* 834(1) (2014) 9-21.
- [9] E. Nouri-Nigjeh, R. Bischoff, A.P. Bruins, H.P. Permentier, Electrochemistry in the mimicry of oxidative drug metabolism by cytochrome P450s, *Current Drug Metabolism* 12(4) (2011) 359-371.
- [10] C. Walgama, R. Nerimetla, N.F. Materer, D. Schildkraut, J.F. Elman, S. Krishnan, A simple construction of electrochemical liver microsomal bioreactor for rapid drug metabolism and inhibition assays, *Analytical Chemistry* 87(9) (2015) 4712-4718.
- [11] S. Shiba, K. Yoshioka, D. Kato, S. Ishihara, H. Anzai, N. Saito, O. Niwa, Electrochemical microfluidic devices for evaluation of drug metabolism, *Journal of Electroanalytical Chemistry* 779 (2016) 86-91.
- [12] A. Fantuzzi, E. Capria, L.H. Mak, V.R. Dodhia, S.J. Sadeghi, S. Collins, G. Somers, E. Huq, G. Gilardi, An electrochemical microfluidic platform for human P450 drug metabolism profiling, *Analytical Chemistry* 82(24) (2010) 10222-10227.
- [13] L. Portychová, K.A. Schug, Instrumentation and applications of electrochemistry coupled to mass spectrometry for studying xenobiotic metabolism: A review, *Analytica Chimica Acta* 993 (2017) 1-21.
- [14] L. Zhu, Y. Shao, H. Xiao, B. Santiago-Schübel, H. Meyer-Alert, S. Schiwy, D. Yin, H. Hollert, S. Küppers, Electrochemical simulation of triclosan metabolism and toxicological evaluation, *Science of the Total Environment* 622-623 (2018) 1193-1201.
- [15] K. Pelivan, L. Frensemeier, U. Karst, G. Koellensperger, B. Bielec, S. Hager, P. Heffeter, B.K. Keppler, C.R. Kowol, Understanding the metabolism of the anticancer drug Triapine: Electrochemical oxidation, microsomal incubation and: In vivo analysis using LC-HRMS, *Analyst* 142(17) (2017) 3165-3176.
- [16] A. Potęga, D. Żelaszczyk, Z. Mazerska, Electrochemical simulation of metabolism for antitumor-active imidazoacridinone C-1311 and in silico prediction of drug metabolic reactions, *Journal of Pharmaceutical and Biomedical Analysis* 169 (2019) 269-278.
- [17] A. Potęga, D. Garwolińska, A.M. Nowicka, M. Fau, A. Kot-Wasik, Z. Mazerska, Phase I and phase II metabolism simulation of antitumor-active 2-hydroxyacridinone with electrochemistry coupled on-line with mass spectrometry, *Xenobiotica* 49(8) (2019) 922-934.
- [18] A. Fantuzzi, M. Fairhead, G. Gilardi, Direct electrochemistry of immobilized human cytochrome P450 2E1, *Journal of the American Chemical Society* 126(16) (2004) 5040-5041.
- [19] V.V. Shumyantseva, A.V. Kuzikov, R.A. Masamrekh, T.V. Bulko, A.I. Archakov, From electrochemistry to enzyme kinetics of cytochrome P450, *Biosensors & Bioelectronics* 121 (2018) 192-204.
- [20] M. Huang, X. Xu, H. Yang, S. Liu, Electrochemically-driven and dynamic enhancement of drug metabolism via cytochrome P450 microsomes on colloidal gold/graphene nanocomposites, *RSC Advances* 2(33) (2012) 12844-12850.
- [21] D. Cui, L. Mi, X. Xu, J. Lu, J. Qian, S. Liu, Nanocomposites of graphene and cytochrome P450 2D6 isozyme for electrochemical-driven tramadol metabolism, *Langmuir* 30(39) (2014) 11833-11840.
- [22] V.V. Shumyantseva, T.V. Bulko, A.V. Kuzikov, R.A. Masamrekh, A.Y. Konyakhina, I. Romanenko, J.B. Max, M. Koehler, A.A. Gilep, S.A. Usanov, D.V. Pergushov, F.H. Schacher, L.V. Sigolaeva, All-electrochemical nanocomposite two-electrode setup for quantification of drugs and study of their electrocatalytical conversion by cytochromes P450, *Electrochimica Acta* 336 (2020).
- [23] J. Tian, J. Wang, Y. Li, M. Huang, J. Lu, Electrochemically Driven Omeprazole Metabolism via Cytochrome P450 Assembled on the Nanocomposites of Ceria Nanoparticles and Graphene, *Journal of the Electrochemical Society* 164(7) (2017) II470-II476.
- [24] M. Sensi, M. del Barrio, C. Baffert, V. Fourmond, C. Leger, New perspectives in hydrogenase direct electrochemistry, *Current Opinion in Electrochemistry* 5(1) (2017) 135-145.
- (**) [25] M. del Barrio, M. Sensi, C. Orain, C. Baffert, S. Dementin, V. Fourmond, C. Leger, Electrochemical Investigations of Hydrogenases and Other Enzymes That Produce and Use Solar Fuels, *Accounts of Chemical Research* 51(3) (2018) 769-777.

This review deals with the methodology adapted to the precise electrochemical characterization of electron transfer from/to hydrogenases as a key to the understanding of catalytic aspects of these enzymes as well as the design of solar fuel cell catalysts.

- [26] E. Anxolabehere-Mallart, C. Costentin, C. Policar, M. Robert, J.-M. Saveant, A.-L. Teillout, Proton-coupled electron transfers in biomimetic water bound metal complexes. The electrochemical approach, *Faraday Discussions* 148 (2011) 83-95.
- [27] G. Cioncoloni, I. Roger, P.S. Wheatley, C. Wilson, R.E. Morris, S. Sproules, M.D. Symes, Proton-Coupled Electron Transfer Enhances the Electrocatalytic Reduction of Nitrite to NO in a Bioinspired Copper Complex, *ACS Catalysis* 8(6) (2018) 5070-5084.
- [28] C. Carreira, M.M.C. dos Santos, S.R. Pauleta, I. Moura, Proton-coupled electron transfer mechanisms of the copper centres of nitrous oxide reductase from *Marinobacter hydrocarbonoclasticus* – An electrochemical study, *Bioelectrochemistry* 133 (2020).
- [29] S.S. Seyedi, M.M. Waskasi, D.V. Matyushov, Theory and Electrochemistry of Cytochrome c, *Journal of Physical Chemistry B* 121(19) (2017) 4958-4967.
- [30] D. Novak, M. Mojovic, A. Pavicevic, M. Zatloukalova, L. Hernychova, M. Bartosik, J. Vacek, Electrochemistry and electron paramagnetic resonance spectroscopy of cytochrome c and its heme-disrupted analogs, *Bioelectrochemistry* 119 (2018) 136-141.
- [31] B. Nguyen, P.L. Gutierrez, MECHANISMS(S) FOR THE METABOLISM OF MITOXANTRONE - ELECTRON-SPIN-RESONANCE AND ELECTROCHEMICAL STUDIES, *Chemico-Biological Interactions* 74(1-2) (1990) 139-162.
- [32] J.R. Ames, P. Kovacic, P.K. Kadaba, P.F. Kiser, ELECTROCHEMISTRY OF ANTICONVULSANTS - ELECTRON-TRANSFER AS A POSSIBLE MODE OF ACTION, *Epilepsia* 33(5) (1992) 936-943.
- [33] N.P. Shetti, S.J. Malode, S.T. Nandibewoor, Electrochemical behavior of an antiviral drug acyclovir at fullerene-C-60-modified glassy carbon electrode, *Bioelectrochemistry* 88 (2012) 76-83.
- [34] J.M.P.J. Garrido, E.M.P.J. Garrido, A.M. Oliveira-Brett, F. Borges, An Electrochemical Outlook on Tamoxifen Biotransformation: Current and Future Prospects, *Current Drug Metabolism* 12(4) (2011) 372-382.
- [35] J.M.P.J. Garrido, E. Quezada, J.L.C. Fajin, M.N.D.S. Cordeiro, E.M.P.J. Garrido, F. Borges, Electrochemical Oxidation of Tamoxifen Revisited, *International Journal of Electrochemical Science* 8(4) (2013) 5710-5723.
- (*) [36] E.A. Hillard, F.C. de Abreu, D.C.M. Ferreira, G. Jaouen, M.O.F. Goulart, C. Amatore, Electrochemical parameters and techniques in drug development, with an emphasis on quinones and related compounds, *Chemical Communications* (23) (2008) 2612-2628.
- A comprehensive overview and rationale of the reactivity of quinones and tamoxifen-based drugs as potent targets of reactive oxygen/nitrogen species*
- [37] M. Muti, M. Muti, Electrochemical monitoring of the interaction between anticancer drug and DNA in the presence of antioxidant, *Talanta* 178 (2018) 1033-1039.
- [38] K. Morawska, T. Poplawski, W. Ciesielski, S. Smarzewska, Electrochemical and spectroscopic studies of the interaction of antiviral drug Tenofovir with single and double stranded DNA, *Bioelectrochemistry* 123 (2018) 227-232.
- [39] N. Chavain, H. Vezin, D. Dive, N. Touati, J.-F. Paul, E. Buisine, C. Biot, Investigation of the redox behavior of ferroquine, a new antimalarial, *Molecular Pharmaceutics* 5(5) (2008) 710-716.
- [40] V. Rep, M. Piskor, H. Simek, P. Misetec, P. Grbcic, J. Padovan, V.G. Markovic, D. Jadresko, K. Pavelic, S.K. Pavelic, S. Raic-Malic, Purine and Purine Isostere Derivatives of Ferrocene: An Evaluation of ADME, Antitumor and Electrochemical Properties, *Molecules* 25(7) (2020).
- [41] S. Realista, S. Quintal, P.N. Martinho, A.I. Melato, A. Gil, T. Esteves, M.D. Carvalho, L.P. Ferreira, P.D. Vaz, M.J. Calhorda, Electrochemical studies and potential anticancer activity in ferrocene derivatives, *Journal of Coordination Chemistry* 70(2) (2017) 314-327.

- [42] P.C.A. Bruijninx, P.J. Sadler, New trends for metal complexes with anticancer activity, *Current Opinion In Chemical Biology* 12(2) (2008) 197-206.
- [43] E. Reisner, V.B. Arion, B.K. Keppler, A.J.L. Pombeiro, Electron-transfer activated metal-based anticancer drugs, *Inorganica Chimica Acta* 361(6) (2008) 1569-1583.
- [44] M. Patra, G. Gasser, The medicinal chemistry of ferrocene and its derivatives, *Nature Reviews Chemistry* 1(9) (2017).
- [45] R.H. Fish, A Bioorganometallic Chemistry Overview: From Cytochrome P450 Enzyme Metabolism of Organotin Compounds to Organorhodium-Hydroxytamoxifen Complexes with Potential Anti-Cancer Properties; A 37 Year Perspective at the Interface of Organometallic Chemistry and Biology, *Australian Journal of Chemistry* 63(11) (2010) 1505-1513.
- [46] V.T.-V. Dao, A.I. Casas, G.J. Maghzal, T. Seredenina, N. Kaludercic, N. Robledinos-Anton, F. Di Lisa, R. Stocker, P. Ghezzi, V. Jaquet, A. Cuadrado, H.H.H.W. Schmidt, *Pharmacology and Clinical Drug Candidates in Redox Medicine, Antioxidants & Redox Signaling* 23(14) (2015) 1113-1129.
- [47] C. Amatore, Basic Concepts, in: M. Baizer, H. Lund (Eds.), *Organic electrochemistry: An introduction and a guide*, New York, 1991.
- (*) [48] A. Jutand, Contribution of electrochemistry to organometallic catalysis, *Chemical Reviews* 108(7) (2008) 2300-2347.
A comprehensive review of the molecular electrochemistry approaches implemented in the determination of the mechanisms in organometallic catalysis.
- [49] E. Labbe, O. Buriez, Fundamental Input of Analytical Electrochemistry in the Determination of Intermediates and Reaction Mechanisms in Electrosynthetic Processes, *Chemelectrochem* 6(16) (2019) 4118-4125.
- [50] D.G. Yakhvarov, A.F. Khusnuriyalova, O.G. Sinyashin, Electrochemical Synthesis and Properties of Organonickel sigma-Complexes, *Organometallics* 33(18) (2014) 4574-4589.
- (*) [51] G. Jaouen, A. Vessieres, S. Top, Ferrocifen type anti cancer drugs, *Chemical Society Reviews* 44(24) (2015) 8802-8817.
A recent review dealing with the historical, therapeutical, and reactivity issues of one of the most developed family of bioorganometallic drugs
- [52] D. Hamels, P.M. Dansette, E.A. Hillard, S. Top, A. Vessieres, P. Herson, G. Jaouen, D. Mansuy, Ferrocenyl Quinone Methides as Strong Antiproliferative Agents: Formation by Metabolic and Chemical Oxidation of Ferrocenyl Phenols, *Angewandte Chemie-International Edition* 48(48) (2009) 9124-9126.
- (**) [53] P. Messina, E. Labbe, O. Buriez, E.A. Hillard, A. Vessieres, D. Hamels, S. Top, G. Jaouen, Y.M. Frapart, D. Mansuy, C. Amatore, Deciphering the Activation Sequence of Ferrociphenol Anticancer Drug Candidates, *Chemistry-a European Journal* 18(21) (2012) 6581-6587.
A seminal paper in the field of the electrochemical characterization of the full oxidation sequence of a bioorganometallic drug.
- [54] J.d.J. Cazares-Marinero, E. Labbe, S. Top, O. Buriez, C. Amatore, G. Jaouen, The effect of protic electron donor aromatic substituents on ferrocenic and 3 ferrocenophanic anilines and anilides: Some aspects of structure-activity relationship studies on organometallic compounds with strong antiproliferative effects, *Journal of Organometallic Chemistry* 744 (2013) 92-100.
- [55] H.Z.S. Lee, O. Buriez, E. Labbé, S. Top, P. Pigeon, G. Jaouen, C. Amatore, W.K. Leong, Oxidative Sequence of a Ruthenocene-Based Anticancer Drug Candidate in a Basic Environment, *Organometallics* 33(18) (2014) 4940-4946.
- [56] H.Z.S. Lee, F. Chau, S. Top, G. Jaouen, A. Vessieres, E. Labbe, O. Buriez, New mechanistic insights into osmium-based tamoxifen derivatives, *Electrochimica Acta* 302 (2019) 130-136.
- [57] J.B. Houston, UTILITY OF IN-VITRO DRUG-METABOLISM DATA IN PREDICTING IN-VIVO METABOLIC-CLEARANCE, *Biochemical Pharmacology* 47(9) (1994) 1469-1479.

