CAHD 2020

sciforum

Proceedings

Redox Behavior of Central-Acting Opioid Tramadol and Its Possible Role in Oxidative Stress †

Uriel Abe Contardi [1], Mateus Morikawa [1] and Douglas Vieira Thomaz [2,*]

[1] Federal University of Technology—Paraná, Curitiba, Brazilurielcontardi@alunos.utfpr.edu.br (U.A.C.); mateusmorikawa@select.eng.br (M.M.)
[2] Federal University of Goiás, Goiânia 74690-900, Brazil
* Correspondence: douglasvthomaz@gmail.com
† Presented at the 1st International e-Conference on Antioxidants in Health and Disease, 01–15 December 2020; Available online: https://cahd2020.sciforum.net/.

Published: 30 November 2020

Abstract: Tramadol (TRA) is a central acting opioid whose biological activities are achieved by interaction with several bodily receptors such as μ-opioid receptors. Considering that central acting drugs may promote oxidative stress, what could lead up to neurodegeneration, this work reported the investigation of the redox behavior of TRA by electrochemical and semi-empirical quantum chemistry approaches (i.e., voltammetry and extended Hückel method—EHM) in order to study TRA prooxidant features. Electrochemical results showcased that TRA exhibited two anodic peaks, namely: 1a at E_{p1a} ≈ +0.03 V and 2a at E_{p2a} ≈ +0.8 V; and a cathodic peak at E_{p1c} ≈ −0.01 V, whereas the quantum chemistry model suggested that the highest occupied molecular orbital n = 0 (HOMO-0) was associated with the tertiary amine in TRA molecule, while HOMO-1 and the lowest unoccupied molecular orbital n = 0 (LUMO-0) were associated with the aromatic benzene ring. The findings were then use to propose an electrooxidation pathway according to the observations and compared to literature, what further offered hints about TRA prooxidant nature. In conclusion, the work herein reported showcases that voltametric and semi-empirical quantum chemistry approaches can be correlated to investigate the redox behavior of CNS-acting compounds.

Keywords: antioxidant; analgesic; neurodegeneration; electrochemistry; quantum chemistry

1. Introduction

Tramadol (TRA) is a central acting opioid whose biological activities are achieved by interaction with several bodily receptors such as μ-opioid receptors [1]. This compound is widely used in medicine to achieve analgesia, and due to its potential for abuse, it is a controlled substance in most countries [2]. Considering that TRA main biological targets are in the central nervous system (CNS), several authors raised concerns about the potential impact of this drug in physicochemical homeostasis. In this sense, literature describes TRA as a pro-oxidant whose chronical use could lead to oxidative stress by stimulating reactive oxygen species (ROS) build up [3].

Although commercially available as a racemic mixture, the core structure of TRA contemplates an aromatic benzene ring with 1,3 substitution (i.e., methoxyl and a 2-((dimethylamino)methyl) cyclohexanol moiety—DMC), as showcased in Figure 1.

The DMC unit showcases a tertiary amine and a tertiary alcohol, and the richness in C-C σ bonds between sp^3 hybridized carbons in this moiety allows some degree of motility (Figure 1). Owing to the good electron accepting and donor activities of aromatic compounds [4], as well as the possibility

of demethylation and genesis of a phenolic moiety in TRA molecule upon excitation, some authors describe that TRA showcases prooxidant capacity [1,3]. Nonetheless, long term use of TRA is often associated to oxidative damage and higher levels of lipid peroxidation biomarkers (i.e., malondialdehyde) [5].

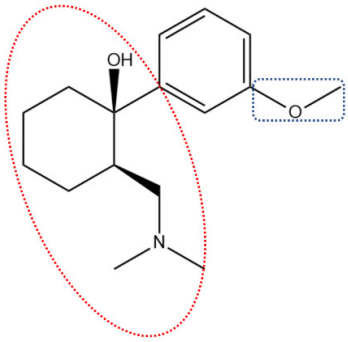

Figure 1. TRA chemical structure showcasing noteworthy moieties, namely: methoxyl unit in the blue-dotted square and the tertiary amine-bearing DMC moiety in the red-dotted ellipse.

Oxidative stress is a major concern for drugs acting in the CNS due to the susceptibility of neurons to ROS [6,7]. Albeit the endogenous antioxidant arsenal is highly effective in mopping up highly energetic species, their build up can eventually overcome the activity of catalases, superoxide dismutase and other reductive enzymes, therefore leading to oxidative stress and damage [4,8]. In this sense, the investigation of the pro-oxidant properties of drug candidates intended for CNS-targeted therapies is of upmost relevance in order to aid the development of effective and safe drugs which could also lead to neuroprotection.

Among the several tools which are used to investigate the physicochemical features of compounds are electrochemistry and theoretical chemistry [9,10]. These approaches allow researchers to gather information regarding the thermodynamics of compounds in several energy levels and reaction coordinates, thence shedding light on the feasibility of chemical transformations such as redox processes [11]. Moreover, reactional kinetics can also be investigated through these tools, thereby allowing a comprehensive study of the physicochemical behavior of compounds.

Nonetheless, several authors employed the electroanalytical approach of voltammetry to study the redox properties of drugs, as well as to correlate the results of ab initio quantum chemistry calculations based on density functional theory [12,13], or semi-empirical approaches consisting of Hartree–Fock formalism such as extended Hückel method (EHM) [14] to better understand the thermodynamics and kinetics involved in oxidation and reduction reactions, as well as to draw interpretations of how chemical compounds might scavenge ROS or promote their formation.

Therefore, this work aimed the investigation of TRA redox behavior at neutral pH (i.e., 7.0) through electrochemical tools and theoretical chemistry calculations in order to better shed light on its reported pro-oxidant properties.

2. Experiments

2.1. Drugs, Solutions and Reagents

TRA chlorhydrate solution (100 mg mL^{-1}); pencil graphite 0.5 mm B; silver filament; potassium phosphate monobasic; potassium phosphate dibasic; potassium ferrocyanide; potassium chloride; potassium chloride solution (3.0 M) saturated with silver chloride; household bleach and distilled water. All salts were purchased from Êxodo científica, Brazil.

2.2. Voltametric Assays

Voltametric investigation was performed in a 5.0 mL electrochemical cell containing 900 μL of phosphate buffered saline (PBS), pH 7.0. TRA solution was diluted until the concentration of 1.0 mg

mL^{-1}, after which a volume of 100 μL was added to the electrochemical cell. The experimental system employed a custom potentiostat/galvanostat coupled to a three-electrode arrange consisting of 0.5 mm B pencil graphite as working electrode; AgCl reference electrode and a stainless-steel counter electrode. The pencil graphite had its lateral surface insulated with electrical tape, while the bottom surface was polished in cellulose paper until and even surface was achieved. The reference electrode was constructed upon treatment of a silver filament with bleach until an even gray-colored cover was visible on the wire surface. Thereafter, the treated wire was placed inside a single open-ended glass container, filled with KCl saturated with AgCl solution, and a porous glass frit was added to the end to make the salt bridge.

The voltametric assay chosen in this work was cyclic voltammetry, due to its fast and reliable execution and optimal performance to gather information of redox processes taking place at the working electrode surface [15]. The experimental parameters for this assay were: start potential of 0 V, anodic and cathodic scan of 100 mVs^{-1} between vertex potentials of −0.8 V and +1.2 V, and stop potential of 0 V.

2.3. Quantum Chemistry Calculations

In this work, the semi-empirical calculation EHM was employed in parallel to the electrochemical assays in order to aid the proposition of an electro-oxidation pathway for TRA [16]. This method was selected due to its fast and easy execution as well as accessibility when compared to ab initio approaches such as density functional theory. The calculations were performed after steric energy minimization through force field approach from classical molecular mechanics, i.e., MM2 method, and also by assisted model building and energy refinement [17,18]. TRA energy-minimized conformer underwent EHM and subsequent rendering of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) in Chem3D® software.

3. Results

In order to evaluate TRA redox behavior at pencil graphite electrode surface, cyclic voltammetries were conducted in triplicates without electrode surface renewal. Results are showcased in Figure 2, wherein a blank assay conducted in triplicates with a clean electrode in PBS, pH 7.0, solution is displayed for comparison.

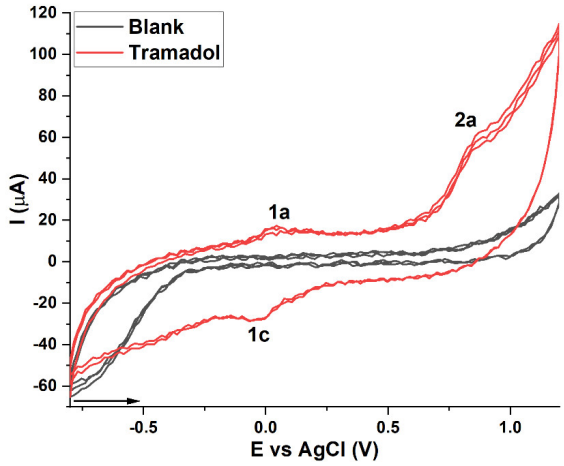

Figure 2. Cyclic voltammogram of TRA. Anodic peaks 1 and 2a suggest the oxidation of TRA at pencil graphite electrode surface, while cathodic peak 1c suggest reduction. The blank assay was performed in 1 mL PBS solution, pH 7.0.

Through cyclic voltammetry, TRA exhibited two anodic peaks, namely: 1a at E_{p1a} ≈ +0.03 V and 2a at E_{p2a} ≈ +0.8 V; and a cathodic peak at E_{p1c} ≈ −0.01 V (Figure 2). The anodic peaks suggest that

electroactive moieties in TRA chemical structure underwent oxidation, while the cathodic peak suggest reduction [19]. Moreover, the faradaic current ratio showcased by peaks 1a and 1c, suggests at least some degree of reversibility, hence $I_{pa}/I_{pc} \approx 1$. Peak 2a, however, did not showcase reversibility (Figure 2) [19].

After the execution of the electrochemical assay, TRA molecule underwent steric energy minimization and quantum calculations by EHM. Results of the rendered model are showcased in Figure 3 and Table 1.

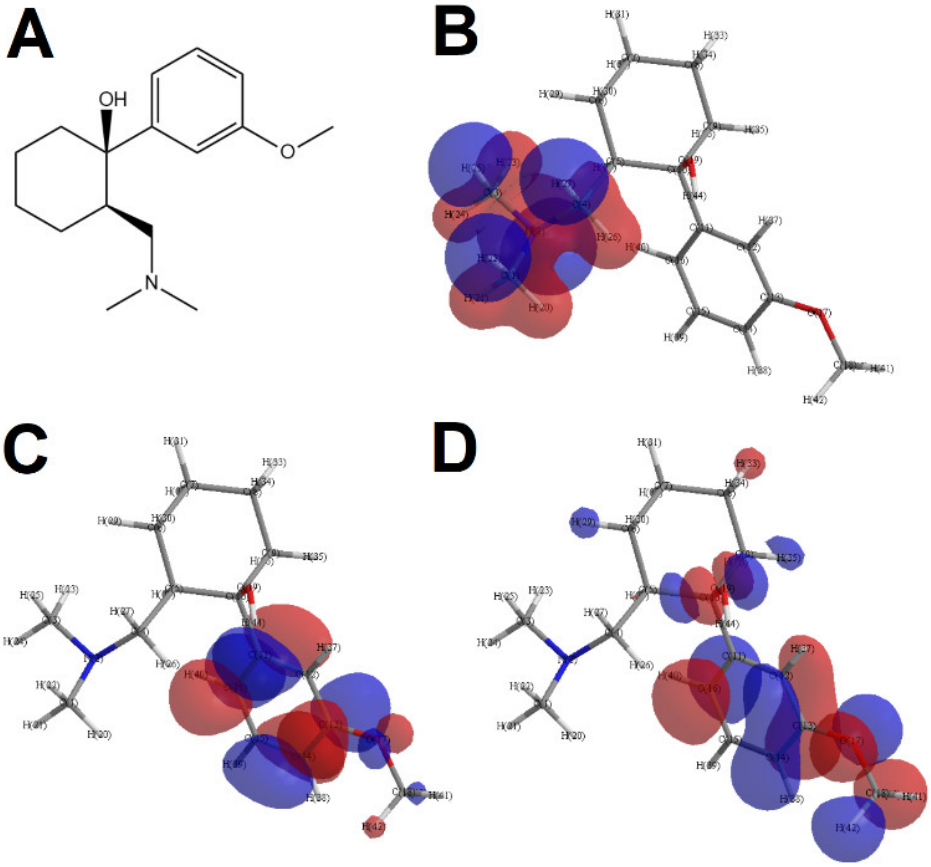

Figure 3. TRA molecule (**A**) and graphical rendering of its HOMO-0 (**B**), LUMO-0 (**C**) and HOMO-1 (**D**). Negative charges are rendered in blue while positive charges are rendered in red.

Table 1. Theoretical energies of HOMO, LUMO and $\Delta E_{LUMO-HOMO\ gap}$ for TRA according to EHM.

Drug	HOMO-n (eV)	LUMO-n (eV)	$\Delta E_{LUMO-HOMO\ gap}$ (eV)
Tramadol	(**n 0**) −9.409	(**n 0**) +0.663	10.072
	(**n −1**) −12.306	(**n +1**) +1.727	14.033
	(**n −2**) −12.443	(**n +2**) +14.701	27.144

Results gathered by EHM suggest that HOMO-0 is associated with the tertiary amine in TRA molecule, while HOMO-1 and LUMO-0 are associated with the aromatic benzene ring (Figure 3B–D). An interesting feature was that in HOMO-1, the rendering of the electric orbital also encompassed the methoxyl moiety (Figure 3D). Considering that smaller energy gaps favor the occurrence redox processes [13], therefore, HOMO-n and LUMO-n can be correlated to the thermodynamic feasibility of oxidation or reduction [14]. In this sense, HOMO-0 and HOMO-1 might be associated with oxidative processes underwent by TRA, while LUMO-0 can be correlated to reduction.

After the electrochemical and quantum chemistry investigation of TRA redox processes, the findings of these techniques were correlated to allow us to propose an electrooxidation pathway for this drug. Results are showcased in Figure 4.

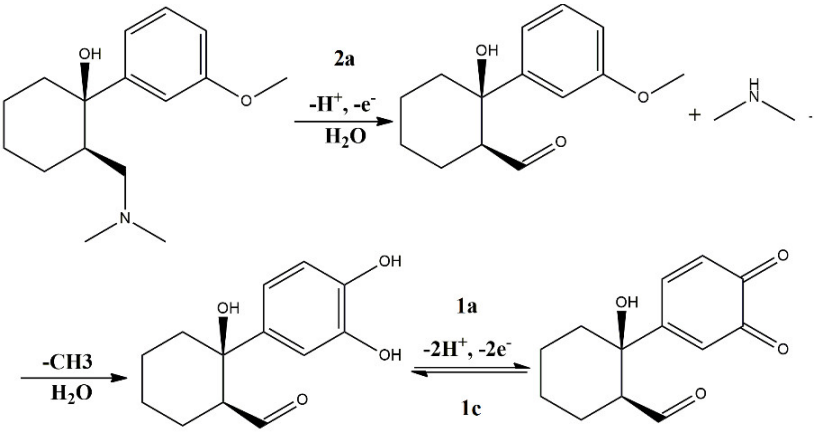

Figure 4. Proposed electrooxidation pathway for TRA molecule.

The proposed electrooxidation showcased in Figure 4 contemplates the irreversible oxidation of the tertiary amine suggested by HOMO-0 graphical rendering, followed by demethylation and the formation of a catechol-quinone system in the aromatic benzene ring wherein HOMO-1 and LUMO-0 renderings were displayed. Each electrooxidation step is marked by its particular faradaic signal, i.e., 2a, 1a and 1c.

4. Discussion

Literature reports that electrochemistry is a valuable approach to investigate redox processes in organic compounds, being voltammetry, the most common technique used to characterize electron transfer phenomena on working electrode surfaces [20,21]. In this sense, we employed cyclic voltammetry to gather information of all redox processes underwent by TRA in the selected electric potential interval. Moreover, the use of pencil graphite electrode (a carbon-based electrode matrix) allowed clear peak visualization, what was previously reported in electroanalytical investigations of other central acting drugs and organic compounds [22].

The anodic and cathodic processes seen at Figure 2 indicate that electroactive moieties in TRA molecule underwent oxidation and reduction, respectively. Considering that the electric current corresponds to the kinetic parameter of a redox reaction seen in a voltammogram, while the electric potential corresponds to the thermodynamic parameter [20], it can be suggested that more energy is needed to promote the oxidation indicated by peak 2a than to promote the oxidation seen at 1a. In addition, peak 2a was clearly higher, therefore suggesting higher kinetics (although capacitive influences cannot be ruled out). Considering that endogenous antioxidants operate at about +0.5 V [7,19], it can be suggested that TRA did not exhibit strong hints of antioxidant capacity, due to its fairly low electrochemical index (I_{p1a}/E_{p1a} + I_{p2a}/E_{p2a}) [19], while the higher amplitude of peak 1c in comparison to 1a suggests better reduction kinetics to this molecule. Furthermore, since aromatic rings exhibit good acceptor-donor behavior, it can be suggested that this moiety could contribute to prooxidant behavior by TRA [14].

The quantum chemistry calculations by EHM showcased that, in the model, HOMO-0 was rendered around the tertiary amine of the DMC moiety, while HOMO-1 and LUMO-0 were rendered in the aromatic benzene ring, being the methoxyl moiety also encompassed in HOMO-1 rendering (Figure 3). Owing to previous reports, wherein is showcased the correlation of HOMO and LUMO renderings and energy gaps to possible oxidation sites in molecules [13,14,23], we therefore suggest that the aforementioned regions are the most thermodynamically feasible to undergo redox reactions.

When analyzing the voltametric and quantum chemistry calculations, there can be suggested that the first oxidation (i.e., HOMO-0) would most likely be in the tertiary amine of DMC moiety, thereby corresponding to peak 2a. Since the first voltametric scan starting at $E = 0$ V did not showcase strong faradaic output where peak 1a would later appear, we therefore suggest 2a as the primary oxidation site. Moreover, the oxidation of amines is well reported in literature regarding voltametric investigations, being of +0.8 V, the most common electric potential value associated to this phenomenon [24,25], which is nonetheless in consonance with our results. Furthermore, considering that the appearance of peaks 1a and 1c suggest a somewhat reversible process, and the HOMO-1 and LUMO-0 were rendered around the aromatic benzene ring, we thence suggest that TRA chemical structure would undergo demethylation and then, the electrosynthesis of a catechol-quinone system [26]. Taking into account that this system is reported to occur at electric potentials close to the ones herein depicted, and also that the proposed electrooxidation of TRA follows a very similar mechanism to other outreaches in literature [27,28], we therefore showcase how voltametric and semi-empirical quantum chemistry approaches can be correlated to investigate the redox behavior of CNS-acting compounds.

5. Conclusions

This work reported the investigation of the redox behavior of TRA by electrochemical and semi-empirical quantum chemistry approaches (i.e., voltammetry and EHM). Electrochemical results showcased that TRA exhibited two anodic peaks and a cathodic peak, whereas the quantum chemistry model suggested that HOMO-0 was associated with the tertiary amine in TRA molecule, while HOMO-1 and LUMO-0 were associated with the aromatic benzene ring. The findings were then use to propose an electrooxidation pathway according to the observations and compared to literature, what further offered hints about TRA prooxidant nature, therefore showcasing how voltametric and semi-empirical quantum chemistry approaches can be correlated to investigate the redox behavior of CNS-acting compounds.

Author Contributions: U.A.B., M.M. and D.V.T. conducted electrochemical experiments, quantum chemistry calculations, analyzed and treated data, as well as wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: Authors would like to thank CAPES-Brazil for the financial support.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TRA	tramadol
CNS	central nervous system
ROS	reactive oxygen species
DMC	2-((dimethylamino)methyl) cyclohexanol
EHM	extended Hückel method
HOMO	highest occupied molecular orbital
LUMO	lowest unoccupied molecular orbital

References

1. Miotto, K.; Cho, A.K.; Khalil, M.A.; Blanco, K.; Sasaki, J.D.; Rawson, R. Trends in Tramadol: Pharmacology, Metabolism, and Misuse. *Anesth. Analg.* **2017**, *124*, 44–51.
2. Das, M.; Jain, R.; Dhawan, A.; Kaur, A. Assessment of abuse liability of Tramadol among experienced drug users: Double-blind crossover randomized controlled trial. *J. Opioid Manag.* **2016**, doi:10.5055/jom.2016.0361.

3. Mohamed, H.M.; Mahmoud, A.M. Chronic exposure to the opioid tramadol induces oxidative damage, inflammation and apoptosis, and alters cerebral monoamine neurotransmitters in rats. *Biomed. Pharmacother.* **2019**, doi:10.1016/j.biopha.2018.11.141.
4. Thomaz, D.V. Flavonoid chemistry and neuroprotection. *Front. Drug, Chem. Clin. Res.* **2020**, *3*, 1–3, doi:10.15761/FDCCR.1000140.
5. Nagakannan, P.; Shivasharan, B.D.; Thippeswamy, B.S.; Veerapur, V.P. Effect of tramadol on behavioral alterations and lipid peroxidation after transient forebrain ischemia in rats. *Toxicol. Mech. Methods* **2012**, doi:10.3109/15376516.2012.716092.
6. Pham-Huy, L.A.; He, H.; Pham-Huy, C. Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* **2008**, *4*, 89.
7. Thomaz, D.V.; Peixoto, L.F.; de Oliveira, T.S.; Fajemiroye, J.O.; da Silva Neri, H.F.; Xavier, C.H.; Costa, E.A.; Alcantara dos Santos, F.C.; de Souza Gil, E.; Ghedini, P.C. Antioxidant and neuroprotective properties of Eugenia dysenterica leaves. *Oxid. Med. Cell. Longev.* **2018**, *2018*, doi:10.1155/2018/8601028.
8. Thomaz, D.V. The Therapeutic Potential of Phytomedicines from Brazilian Cerrado Herbs against Neurodegenerative Diseases. *Am. J. Biomed. Sci. Res.* **2020**, *7*, 374–377, doi:10.34297/AJBSR.2020.07.001180.Received.
9. Zinola, C.F. Density functional theory. In *Electrocatalysis: Computational, Experimental, and Industrial Aspects*; CRC Press: Boca Raton, FL, USA, 2010; ISBN 9781420045451.
10. Deepa, P.; Kolandaivel, P.; Senthilkumar, K. Theoretical investigation of interaction between psoralen and altretamine with stacked DNA base pairs. *Mater. Sci. Eng. C* **2012**, *32*, 423–431, doi:10.1016/J.MSEC.2011.11.014.
11. Rodrigues, E.S.B.; de Macêdo, I.Y.L.; da Silva Lima, L.L.; Thomaz, D.V.; da Cunha, C.E.P.; Teles de Oliveira, M.; Ballaminut, N.; Alecrim, M.F.; Ferreira de Carvalho, M.; Isecke, B.G.; et al. Electrochemical Characterization of Central Action Tricyclic Drugs by Voltammetric Techniques and Density Functional Theory Calculations. *Pharmaceuticals* **2019**, *12*, 116, doi:10.3390/ph12030116.
12. El-Gogary, T.M.; Koehler, G. Interaction of psoralens with DNA-bases (II): An ab initio quantum chemical, density functional theory and second-order MØller-Plesset perturbational study. *J. Mol. Struct. THEOCHEM* **2009**, *895*, 57–64, doi:10.1016/J.THEOCHEM.2008.10.012.
13. Rodrigues, E.S.B.; de Macêdo, I.Y.L.; da Silva Lima, L.L.; Thomaz, D.V.; da Cunha, C.E.P.; Teles de Oliveira, M.; Ballaminut, N.; Alecrim, M.F.; Ferreira de Carvalho, M.; Isecke, B.G.; et al. Electrochemical characterization of central action tricyclic drugs by voltammetric techniques and density functional theory calculations. *Pharmaceuticals* **2019**, doi:10.3390/ph12030116.
14. Thomaz, D.V.; de Oliveira, M.G.; Rodrigues, E.S.B.; da Silva, V.B.; Santos, P.A. Dos Physicochemical investigation of psoralen binding to double stranded dna through electroanalytical and cheminformatic approaches. *Pharmaceuticals* **2020**, doi:10.3390/ph13060108.
15. Climent, V.; Feliu, J.M. Cyclic voltammetry. In *Encyclopedia of Interfacial Chemistry: Surface Science and Electrochemistry*; Elsevier: Amsterdam, The Netherlands, 2018; ISBN 9780128098943.
16. Matito, E.; Feixas, F.; Solà, M. Electron delocalization and aromaticity measures within the Hückel molecular orbital method. *J. Mol. Struct. THEOCHEM* **2007**, doi:10.1016/j.theochem.2007.01.015.
17. Ponder, J.W.; Richards, F.M. An efficient newton-like method for molecular mechanics energy minimization of large molecules. *J. Comput. Chem.* **1987**, doi:10.1002/jcc.540080710.
18. Case, D.A.; Cheatham, T.E.; Darden, T.; Gohlke, H.; Luo, R.; Merz, K.M.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R.J. The Amber biomolecular simulation programs. *J. Comput. Chem.* **2005**, *26*, 1668–1688.
19. Leite, K.C.D.S.; Garcia, L.F.; Lobón, G.S.; Thomaz, D.V.; Moreno, E.K.G.; Carvalho, M.F.D.; Rocha, M.L.; Santos, W.T.P.D.; Gil, E.D.S. Antioxidant activity evaluation of dried herbal extracts: An electroanalytical approach. *Braz. J. Pharmacogn.* **2018**, *28*, doi:10.1016/j.bjp.2018.04.004.
20. Thomaz, D.V.; Filho, A.M.D.A.; Macedo, I.Y.L.; Rodrigues, E.S.B.; Gil, E.D.S. Predictive Modelling to Study the Electrochemical Behaviour of PdO, TiO_2 and Perovskite-Type $LaFeO_3$ Modified Carbon Paste Electrodes. *Path Sci.* **2019**, *5*, 4001–4007, doi:10.22178/pos.45-3.
21. Mendoza, S.; Bustos, E.; Manríquez, J.; Godínez, L.A. Voltammetric Techniques. In *Agricultural and Food Electroanalysis*; John Wiley & Sons, Ltd: Hoboken, NJ, USA, 2015; ISBN 9781118684030.
22. David, I.G.; Popa, D.E.; Buleandra, M. Pencil graphite electrodes: A versatile tool in electroanalysis. *J. Anal. Methods Chem.* **2017**, *2017*, doi:10.1155/2017/1905968.

23. Jabeen, H.; Saleemi, S.; Razzaq, H.; Yaqub, A.; Shakoor, S.; Qureshi, R. Investigating the scavenging of reactive oxygen species by antioxidants via theoretical and experimental methods. *J. Photochem. Photobiol. B Biol.* **2018**, *180*, 268–275, doi:10.1016/J.JPHOTOBIOL.2018.02.006.
24. Thomaz, D.V.; de Oliveira, M.T.; Lobón, G.S.; da Cunha, C.E.P.; Machado, F.B.; Moreno, E.K.G.; Leite, K.C.S.; Ballaminut, N.; Alecrim, M.F.; de Carvalho, M.F.; et al. Development of Laccase-TiO_2@carbon paste biosensor for voltammetric determination of paracetamol. *Int. J. Electrochem. Sci.* **2018**, *13*, doi:10.20964/2018.11.61.
25. Antunes, R.S.; Thomaz, D.V.; Garcia, L.F.; Gil, E.D.S.; Sommerset, V.S.; Lopes, F.M. Determination of Methyldopa and Paracetamol in Pharmaceutical Samples by a Low Cost Genipa americana L. Polyphenol Oxidase Based Biosensor. *Adv. Pharm. Bull.* **2019**, *9*, 416–422, doi:10.15171/apb.2019.049.
26. DuVall, S.H.; McCreery, R.L. Control of catechol and hydroquinone electron-transfer kinetics on native and modified glassy carbon electrodes. *Anal. Chem.* **1999**, doi:10.1021/ac990399d.
27. Lütke Eversloh, C.; Schulz, M.; Wagner, M.; Ternes, T.A. Electrochemical oxidation of tramadol in low-salinity reverse osmosis concentrates using boron-doped diamond anodes. *Water Res.* **2015**, doi:10.1016/j.watres.2014.12.021.
28. Zimmermann, S.G.; Schmukat, A.; Schulz, M.; Benner, J.; Gunten, U.V.; Ternes, T.A. Kinetic and mechanistic investigations of the oxidation of tramadol by ferrate and ozone. *Environ. Sci. Technol.* **2012**, doi:10.1021/es203348q.

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).