Electrochemical oxidation of artificial and natural hydroxyl containing compounds in aprotic organic solvents

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

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AND NATURAL HYDROXYL CONTAINING
COMPOUNDS IN APROTIC ORGANIC SOLVENTS**

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SCHOOL OF PHYSICAL & MATHEMATICAL SCIENCES
NANYANG TECHNOLOGICAL UNIVERSITY

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Aprotic Organic Solvents**

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School of Physical and Mathematical Sciences

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
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Abstract

Electrochemical oxidation studies were performed in aprotic organic solvents on two types of artificial hydroxyl containing emerging contaminants; namely bisphenol A and phenolic benzotriazoles (UV234 and UV327) as well as on the natural hydroxyl containing vitamin D compounds (vitamins D₃ and D₂). The techniques utilized to study the compounds included CV, CPE as well as combining spectroscopic methods such as UV-vis and EPR with electrochemistry and chemical oxidation. All 3 types of hydroxyl containing compounds were found to undergo chemically irreversible oxidation processes on the short voltammetric timescale, indicating that the initial heterogeneous electron transfer steps produced oxidized intermediates that were not long-lived. Further investigations enabled the determination of the exact electron transfer and chemical steps involved in the oxidation process. In this thesis, the oxidative mechanism for the compounds are proposed along with the identity of the intermediate species and final oxidized products which can provide useful insights into the reactions that can occur in lipophilic environments during oxidative metabolism as well as highlight the possible role of the intermediate species as reactive metabolites in oxidative degradation pathways.

The electrochemical oxidation of the two artificial phenolic classes of compounds occurred at the phenolic moiety in a mechanism that is similar to phenols in general, while the electrochemical oxidation of the natural hydroxyl containing vitamin D most likely occurred at the triene moiety. Bisphenol A is chemically oxidized *via* a four-electron/two-proton process ($-4e^-/-2H^+$) in a $2 \times$ ECE mechanism to form a relatively unstable dication which reacts quickly in the presence of water in acetonitrile. UV234 and UV327 displayed similar behavior and can be electrochemically oxidized *via* a two-electron/one-proton process ($-2e^-/-1H^+$) in an ECE mechanism under neutral and acidic conditions. In basic conditions, the phenolic benzotriazole is electrochemically oxidized

in a CEC_{dim} process to form neutral benzotriazolyl-substituted phenoxyl radicals that immediately undergo a reversible dimerization (C_{dim} -step) to form a dimer which exists in equilibrium with the radicals. Vitamin D is electrochemically oxidized in an ECCE mechanism initially *via* one-electron to form a cation radical, which reacts homogeneously in two chemical steps [where one chemical step is fast (< 1 s) and one is relatively slow (> 1 s)]. On the longer electrolysis timescale, the oxidized product undergoes a second one-electron transfer at a less positive potential.

Surface coverage of drop cast nanomaterial coatings formed on electrodes were assessed after investigations at a MWCNT-coated GC electrode suggested that electrode modification with nanomaterials was not useful in resolving the electrode fouling problem caused by adsorption of oxidized products during oxidation. It was demonstrated that the drop casting procedure is unable to prevent solution phase ions from diffusing through the nanomaterial coating to the base electrode and the contribution from the base electrode on the current-potential trace must be taken into account to fully understand the current response.

List of Publications

The results in this thesis project are published in the following research articles.

Research Articles:

CHAPTER 3

Chan, Y. Y.; Yue, Y.; Li, Y.; Webster, R. D. Electrochemical/Chemical Oxidation of Bisphenol A in a Four-Electron/Two-Proton Process in Aprotic Organic Solvents. *Electrochim. Acta* **2013**, 112, 287-294.

CHAPTER 5

Chan, Y. Y.; Yue, Y.; Webster, R. D. Voltammetric Studies on Vitamins D₂ and D₃ in Organic Solvents. *Electrochim. Acta* **2014**, 138, 400-409.

CHAPTER 6

Chan, Y. Y.; Eng, A. Y. S.; Pumera, M.; Webster, R. D. Assessments of Surface Coverage after Nanomaterials are Drop Cast onto Electrodes for Electroanalytical Applications. *ChemElectroChem* **2015**, 2, 1003-1009.

Scientific Conference Poster:

Chan, Y. Y.; Webster, R. D (2013). Electrochemical Oxidation of Bisphenol A. In: 224th Electrochemical Society Meeting, San Francisco, USA.

Index of Abbreviations

BPA	Bisphenol A or 2,2-bis(4-hydroxyphenyl)propane
BZTs	Benzotriazoles
C step	Chemical step
C _{dim} step	Chemical (dimerization) step
CNT	Carbon nanotubes
CPE	Controlled potential electrolysis
CV	Cyclic voltammetry/cyclic voltammogram
DCM	Dichloromethane
DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
E step	Electron transfer step
ECE	Electron transfer – Chemical – Electron transfer (mechanism steps)
EPR	Electron paramagnetic resonance
Fc/Fc ⁺	Ferrocene/Ferricenium redox couple
GC	Glassy carbon electrode
LSV	Linear sweep voltammetry
mM	Millimolar
mV	Millivolt
MWCNT	Multiwalled carbon nanotubes
NO ⁺	Nitrosonium cation
NMR	Nuclear magnetic resonance
RDE	Rotating disk electrode
SEM	Scanning electron microscopy
SWV	Square-wave voltammetry
TCNQ	7,7,8,8-tetracyanoquinodimethane
UV-vis	Ultraviolet-visible
UV234	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol
UV327	2,4-di-tert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol

Index of Symbols

$^{\circ}\text{C}$	Degree centigrade
D	Diffusion coefficient
E^0	Formal electrode potential
E_{λ}	Switching potential
$E_{1/2}$	Half-wave potential
E_p^{ox}	Oxidative peak potential (anodic)
E_p^{red}	Reductive peak potential (cathodic)
ΔE_{pp}	Peak-to-peak separation
h	Hour (time)
i_p^{ox}	Oxidative peak current (anodic)
i_p^{red}	Reductive peak current (cathodic)
k_b	Rate constant for backward reaction
k_f	Rate constant for forward reaction
K_{eq}	Equilibrium constant
min	Minute (time)
s	Second (time)

Chapter 1

Introduction

1.1 Biotransformation of Artificial and Natural Hydroxyl Containing Compounds

1.2 Artificial and Natural Hydroxyl Containing Compounds

1.2.1 Artificial hydroxyl containing compounds I: Bisphenol A

1.2.2 Artificial hydroxyl containing compounds II: Phenolic Benzotriazoles

1.2.3 Natural hydroxyl containing compound: Vitamin D

1.3 Investigation of Oxidized Metabolites

1.3.1 Current analytical methods used for detection of oxidative metabolites

1.3.2 Electrochemical methods used for detection of oxidative metabolites

1.4 Rationale and Aim of Thesis

1.4.1 Using electrochemical methods for studying metabolic reaction mechanisms

1.4.2 Performing experiments in aprotic organic solvents

1.4.3 Aim of thesis

References

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1.1 Biotransformation of Artificial and Natural Hydroxyl Containing Compounds

1.1.1 *Metabolism of artificial and natural compounds in the human body*

Compounds that enter the body are usually absorbed, distributed, and processed by undergoing a series of biological processes and chemical transformations where they are metabolized, stored and excreted depending on the nature of the compounds. Nutrient metabolism activates naturally occurring nutrients (macronutrients such as carbohydrates, protein, triglycerides and micronutrients such as vitamins) into biologically active forms to be stored and used for biological functions important to human health while the detoxification metabolism deactivates artificial xenobiotics (such as drugs and environmental contaminants) for removal from the body.¹

Metabolism constitutes of essential enzyme-catalyzed biotransformation reactions which involve oxidation and reduction steps occurring within the cells of living organisms that are vital to sustain life. The metabolic pathways balance between catabolism (oxidation process; where important nutrients are digested to provide energy) and anabolism (reduction process; where energy is consumed to synthesize new cells) to support the maintenance of metabolic processes and regulate physiological homeostasis. The human liver cells provide a protective mechanism that can tolerate moderate amounts of harmful compounds. It responds with a detoxification metabolism which involves mainly oxidation reactions to eliminate a wide range of exogenous foreign chemicals as well as endogenous compounds that are produced in excess as byproducts from normal metabolic activity.

Detoxification metabolism is however adversely affected by the presence of excessively high concentration of toxins including overdose of xenobiotics or abnormal high levels of natural compounds such as fatty acids, eicosanoids, steroid hormones and

fat soluble vitamins caused by poor nutritional knowledge or metabolic dysfunction.² Important nutrients such as vitamins A and D that perform fundamental roles in the maintenance of health can also result in toxicity when overconsumed.³ Toxicity effects arise especially when the concentration of the toxins exceeds the tolerance level of the detoxification system. As the deactivation pathways become saturated and the body grows incapable of coping with the high toxins level, metabolism may be directed towards the activation pathway instead, causing the body to succumb to toxicity. It is also possible that some compounds are metabolized into even more reactive and potentially more toxic metabolites during the enzyme-catalyzed biotransformation reactions.^{4,5}

It is imperative to study how many artificial xenobiotics and natural nutrients are converted in the body in order to evaluate the possible risk of the toxicity effects to human health. A detailed understanding of the reaction mechanism that the compounds undergo during the metabolic pathways will be helpful in investigating the current controversy that exists regarding the concentration limit at which these compounds cause toxicity and whether a regulatory action for minimizing the chemical exposure of these chemicals should be implemented.

1.1.2 Oxidative metabolism of artificial and natural hydroxyl containing compounds

The presence of a hydroxyl group ($-OH$) either in the form of an aliphatic alcohol or as a phenol functional group is common in a wide variety of artificial and natural compounds. The hydroxyl group increases the polarity of the compounds and can serve as a hydrogen bond donor and acceptor while maintaining its neutral charge at physiological pH.

Despite the presence of hydroxyl groups, there are some artificial foreign chemicals and naturally occurring fat soluble vitamins which are lipophilic making them

difficult for excretion so metabolism is required to convert the substrates to more polar and hydrophilic derivatives. The biochemical reactions for metabolism occur *via* 2 phases that can take place singly but are more often in sequence. Phase I metabolism involves activating the substrate by the introduction of a polar functionality with oxidation, reduction or hydroxylation reactions mediated with oxidative enzymes. Phase II metabolism involves a subsequent conjugation with a large hydrophilic endogenous molecule like glutathione, glucuronide or sulfate. The phase II conjugation reactions are important for detoxification as it increases the solubility of the products to facilitate excretion through the bile or urine.

The major pathway for the metabolism of hydroxyl groups is oxidative metabolism which is carried out by the oxidative enzymes located predominantly in the smooth endoplasmic reticulum of the hepatocytes in the liver, although they are also found distributed widely in the tissues. The enzymes involved in the oxidative metabolism of hydroxyl containing compounds are alcohol dehydrogenases (ADH), cytochrome P450 enzymes (CYP450s) and prostaglandin H synthase (PGHS).^{7,8} Other than catalyzing the oxidation from alcohols to aldehydes, ketones or carboxylic acids intermediates, the major oxidative enzymes CYP450s also catalyze a wide range of phase I oxidation reactions namely hydroxylation, epoxidation, sulfonation, N-, O- and S-dealkylation, dehalogenation and deamination.^{8,9}

Besides mediating the oxidative metabolism of xenobiotics for deactivation and eliminating them from the body, the oxidative enzymes are also involved in the biosynthesis and metabolism of natural endogenous compounds. They play biologically significant roles in maintaining a dynamic balance between steroid activation (converting to active forms before they execute their biological responses) and inactivation (termination of the active forms to prevent toxicity from accumulation of high levels) to

regulate cellular proliferation and differentiation, reproductive development, and water and electrolyte homeostasis.¹⁰⁻¹²

1.1.3 Oxidation of hydroxyl containing compounds to reactive metabolites during oxidative metabolism

The natural defense system led by both (phase I) oxidative and (phase II) conjugative metabolism facilitates detoxification by deactivating the hydroxyl containing compounds into glucuronated, sulfated and methylated metabolites. However, there are some chemicals that can be activated *via* oxidative metabolism before they proceed with conjugation reactions. Instead of going through the detoxification pathway, they are metabolically activated into highly reactive metabolites that can exhibit even stronger toxic adverse effects than their parent compounds.^{4,5}

Endocrine disrupting activity has been associated with a group of hydroxyl containing compounds and their metabolites including phenolic emerging environmental contaminants bisphenols used in food contact materials, triclosan in antibacterials, alkylphenols used as surfactants, synthetic phenolic antioxidants as well as synthetic estrogens used as contraceptives (ethinyl estradiol and diethylstilbestrol) and natural occurring estrogens (estrone and estradiol).¹³⁻¹⁸ Toxicity has been reported from hypervitaminosis that mostly affect fat soluble hydroxyl containing vitamins since they are stored longer in the liver than water soluble vitamins. Excessive amounts of vitamin D metabolites can cause hypercalcemia and abnormal high storage levels of retinoids (retinol and its metabolites) forms of vitamin A can result in bone loss that leads to osteoporosis.¹⁹⁻²¹

There are cases where the hydroxyl containing compounds can be oxidized into electrophilic metabolites and free radicals *via* oxidative metabolism. Primary aliphatic alcohols are oxidized into reactive electrophilic intermediates such as aldehydes while

phenols are oxidized to quinones, quinonemethides or quinonimines. It is possible that the reactive metabolites can still be trapped by the endogenous conjugating molecules for detoxification provided that the deactivation pathways are not saturated and the conjugating molecules are not depleted. Otherwise, the oxidized metabolites which are highly reactive electrophilic species can bind directly to nucleophilic macromolecules such as amino acids and DNA in the body to cause adverse effects. The irreversible modification to the biological macromolecules *via* covalent bonding reportedly disrupts a wide range of important cellular functions and causes serious damage to cells and tissues.²²⁻²⁶

They are also capable of inducing and inhibiting the expressions of CYP450s. Reactive metabolites can bind covalently to the CYP450s enzymes and irreversibly inhibit the enzyme activity. They can induce and cause an overexpression of the CYP450s, disrupting normal endocrine functions and steroid hormones levels by upsetting the balance between steroidogenesis and metabolism.²²⁻²⁴ Reactive metabolites also uncouple the catalytic reaction of CYP450s and lead to the generation of highly reactive oxygen species namely hydrogen peroxide (H_2O_2), hydroxyl radicals ($\text{OH}^\bullet$) and superoxide anions ($\text{O}_2^{\bullet-}$) that seek to attack biological macromolecules especially when the usual deactivation pathways are overloaded. The radicals damage unsaturated fatty acids leading to lipid peroxidation and destruction of the cellular structure. When the cells are unable to cope with the increasing generation of the reactive oxygen species formed from either endogenous or exogenous cellular redox imbalance, they trigger oxidative stress causing an oxidative damage to the DNA and cellular necrosis. Reactive oxygen species and increased levels of oxidative stress have been linked to chronic inflammatory diseases such as carcinogenesis, atherosclerosis, diabetes and neurodegeneration.²⁵⁻²⁷

As the fate of the chemicals in the body is determined largely by the CYP450s-catalyzed activation and deactivation pathways involved during oxidative metabolism

(Scheme 1.1), investigation on the identity of the possible oxidized electrophilic metabolites formed becomes an important determinant in assessing the toxicity effects of the artificial and natural hydroxyl containing compounds. It is necessary to study the detailed reaction mechanisms including the identification of the intermediates and the final degraded products formed during their oxidative metabolic pathways in order to gain a better understanding of their conversion in the body.

Scheme 1.1. Activation and deactivation pathways in oxidative metabolism.

1.1.4 Oxidation of hydroxyl containing compounds to reactive transformation products during oxidative water treatment

Due to their persistency and lipophilic nature, many xenobiotic compounds are resistant to degradation with the current existing conventional water treatment processes. Oxidative water treatment methods such as pure ozonation, ozonation combined with

hydrogen peroxide and/or UV light and chemical oxidants such as potassium permanganate and ferrate are most often used to treat wastewater but studies have showed either incomplete removal of contaminants or detection of oxidized transformation products produced during water treatment processes that use oxidation. It is possible that the harsh oxidation method leads to the formation of oxidized transformation products that exhibit even more toxic and stronger endocrine disrupting properties than the original compounds of the emerging phenolic environmental contaminants.²⁸⁻³⁵

The transformation products produced from the oxidation of hydroxyl containing environmental contaminants are of immense concern especially if they are biologically active or resistant to biodegradation as they can enter the water sources and bioaccumulate in the environment. This is highlighted with the fact that in contrary to performing the intended task of purifying water, the employment of oxidation techniques during water treatment processes are introducing persistent oxidative products of toxicological concern.

1.2 Artificial and Natural Hydroxyl Containing Compounds

Over the recent decades, there is an increasing presence of potentially hazardous chemicals and their metabolites due to the increased production of chemicals as a result of technological advancement and industrialization. Even though they exist in low concentration as either free or conjugated forms in the wastewater and surface waters,^{28,36-39} with trace amounts detected in human tissues,⁴⁰⁻⁴² reports have shown that they have the potential to bioaccumulate and concentrate in the environment which can have a detrimental impact on human health and cause widespread prevalence of diseases.

There is an extensive list of emerging contaminants that are currently investigated for regulatory action by the U.S. Environmental Protection Agency (EPA).⁴³ These potential environmental contaminants are either new chemicals that have been synthesized recently or chemicals that have been around for a long time but are only detected due to advanced detection techniques in recent years. They span over a broad range of artificial chemicals used daily in personal care and household consumer products, antiseptics, insect repellents and pharmaceuticals.

In this thesis, we study two artificial phenolic emerging contaminants namely bisphenol A which is commonly found in food contact plastics and phenolic benzotriazoles which are used as UV-stabilizers in many household products. We also study the natural hydroxyl containing vitamin D compounds which can behave both as a hormone essential for the maintenance of calcium and bone homeostasis and yet also a toxin if consumed excessively.

1.2.1 Artificial hydroxyl containing compounds I: Bisphenol A

1.2.1.1 Background of bisphenol A

Bisphenol A (BPA) or 2,2-bis(4-hydroxyphenyl)propane is a high production volume synthetic chemical that is synthesized by the condensation reaction of phenol and acetone in the presence of a catalyst. BPA contains two phenol functional groups connected by a bridge containing two methyl groups as illustrated in Scheme 1.2. It is widely used as a monomer in the production of polycarbonate and polysulfone plastics (used in food contact materials such as baby feeding bottles, microwave ovenware and reusable plastic water containers) and epoxy resins (used as inner protective linings for canned food and beverages).⁴⁴ An incomplete polymerization during the manufacturing process or hydrolysis of the ester bonds between the BPA monomers from repeated heat exposure and usage can result in a migration from the coatings on the container into the food and the environment.⁴⁵ There are many reports available in the literature that provided evidence of widespread human exposure and the release of BPA into the environment. Substantial amounts of the compound have been detected in human biological fluid samples likely to be ingested through food and water due to BPA leaching⁴⁶⁻⁴⁹ as well as in surface waters and sediment⁵⁰⁻⁵² and soil samples.⁵³

Scheme 1.2. Chemical structure of 2,2-bis(4-hydroxyphenyl)propane (bisphenol A).

1.2.1.2 Adverse health effects associated with exposure to bisphenol A

There are increasing concerns regarding the potential toxicity and hormonal activities of BPA because of emerging evidence of adverse health effects reported in

laboratory animal studies. Animal studies on rats, aquatic organisms and wildlife have shown evidence of reproductive and developmental toxicity especially in early postnatal stages due to their reduced capacity in metabolism but further studies are needed to translate the findings from animal studies to toxic effects on human health.^{47,48,52,54} BPA is a known endocrine disruptor which mimics hormones and is capable of disrupting normal hormonal functions and metabolic pathways, but the biochemical mechanism through which it acts as an endocrine disruptor remains unclear.⁴⁵ There is also no consensus on the maximum exposure level that will pose a health risk.

This potentially toxic pollutant was first banned in 2010 by Canada, followed by countries in Europe and in July 2012, the Food and Drug Administration (FDA) outlawed the use of BPA in baby bottles but not in other general consumer products. BPA is poorly soluble in water; the compound has an octanol-water partition coefficient, log K_{ow} value of 3.64⁴⁷ suggesting slightly higher potential for bioaccumulation in the organic carbon of sediments and the tendency to reside and accumulate in lipid bilayers, concentrating in aquatic food chains. As of 2008, BPA is classified as an important emerging contaminant and is currently monitored for its potential toxicity and persistence in the environment by many international agencies.^{43,47}

1.2.1.3 Metabolic pathways of bisphenol A in biological systems

Upon entering the body, the biologically active BPA is rapidly absorbed from the gastrointestinal tract and undergoes metabolism in the gut wall and liver. BPA is metabolized primarily *via* phase II detoxification pathways. The BPA molecule undergoes conjugation with glucuronic acid (glucuronidation) in the liver catalyzed by the enzyme UGT (UDP-glucuronosyltransferase) to form an inactive metabolite, bisphenol A glucuronide. It is believed that the BPA glucuronide does not bind to estrogen receptor and is removed from the blood by the kidneys followed by a rapid excretion since it is

soluble in water^{47,48,55} but there are reports showing that a reversible reaction is possible which releases the free BPA again.⁵⁶ Volkel *et al.* have reported that BPA is eliminated in less than 6 hours *via* glucuronidation in humans,⁵⁵ indicating that BPA is less persistent than other endocrine disruptors such as the banned chemical dichlorodiphenyltrichloroethane (DDT) used as a main ingredient in insecticides. Other minor metabolites that have been frequently identified in laboratory rat studies include bisphenol A sulfate and hydroxybisphenol A⁵⁷ as well as glutathione conjugates, bisphenol A dimers or cleaved molecules.⁵⁸ Recently, Liao and Kannan reported the determination of bisphenol A glucuronide and disulfate in human serum for the first time.⁵⁹ Studies have shown evidence of BPA generating reactive oxygen species *via* the cytochrome P450 pathway, which decreases the activity of antioxidant enzymes and increases lipid peroxidation by inducing oxidative stress, eventually causing oxidative cell damage.^{58,60-62}

1.2.1.4 Literature precedence on possible oxidative metabolites

It is believed that BPA can undergo oxidative metabolic routes to form metabolites that interact irreversibly with biological molecules which can induce toxicity. Yoshihara *et al.*^{63,64} incubated BPA with human and several animal liver S9 fractions and identified 4-methyl-2,4-bis(*p*-hydroxyphenyl)pent-1-ene (MBP) as an active estrogenic metabolite, likely formed from a recombination of a one-electron oxidation product of the carbon-phenyl bond cleavage. The metabolite exhibited approximately 500 times more potent estrogenic activity than BPA in several *in-vitro* assays. Atkinson and Roy reported the discovery of an oxidative metabolite, bisphenol *o*-quinone which can bind to DNA using a monoquinone peroxidase activation system and microsomal cytochrome P450 mono-oxygenases *in vitro*.⁶⁵ Yoshida *et al.*^{66,67} isolated two oxidized products of BPA namely the monoquinone and bisquinone using a radical oxidant. Both suggested that the

quinine products are likely formed from an initial hydroxylation step which subsequently undergoes an oxidation reaction.⁶⁵⁻⁶⁷ Several other BPA degradation intermediates and products have also been identified along with the proposal of a few similar reaction pathways during the electrolysis of millimolar concentrations of BPA in water^{68,69} and oxidation using ozone⁷⁰ and ferrate⁷¹ in an effort to explore potential water treatment methods, but BPA and its potential metabolites are likely to react differently in organic and aqueous solvents.

Despite the extensive work conducted on the metabolism and toxicity of BPA, there are considerable discrepancies in the outcomes, with respect to the type of metabolites generated during biotransformation, the adverse health effects observed and the level at which these effects occur. There is controversy about the safety of BPA as the mechanism by which BPA causes toxicity remains unclear.

1.2.2 Artificial hydroxyl containing compounds II: Phenolic Benzotriazoles

1.2.2.1 *Background of phenolic benzotriazoles*

Phenolic benzotriazoles (BZTs), also known as benzotriazole ultraviolet (BUVs) stabilizers or Tinuvin, are an important class of UV absorbers designed to absorb strongly at wavelengths between 280 – 400 nm where most materials are susceptible to degradation on exposure to solar radiation.^{72,73} They are commonly used as an additive to a wide variety of consumer and industrial products such as polymers, films, paints, personal care products and food contact materials to protect against photodegradation. Phenolic BZTs are high production volume synthetic chemicals that contain a phenolic functional group attached to the benzotriazole moiety, the compounds vary in substituents (alkyl, aryl or ester functional groups) at the ortho and para positions of the phenol designed to meet specific performance criteria, of which UV-P, UV-234, UV326, UV-327 and UV-328 are often used (Scheme 1.3). Unlike the benzotriazole molecules which are used for anticorrosive applications, the additional phenolic group attached to BZTs can provide UV stabilization *via* an enol-keto tautomerism as shown in Scheme 1.3. The photostabilization mechanism is based on a reversible proton transfer that takes place in picoseconds which involves intramolecular hydrogen bonding between the hydroxyl group of the phenyl ring and the nitrogen atom of the benzotriazole moiety during the absorption of a photon and then dissipating the absorbed energy to rapidly quench the excited state back to the ground state.^{72,73}

Scheme 1.3. Photostabilization mechanism for selected phenolic benzotriazoles.

1.2.2.2 Adverse health effects associated with exposure to phenolic benzotriazoles

As they are not bound covalently to the polymers, leaching from the products to the environment can occur. Phenolic BZTs are highly lipophilic compounds. The octanol-water partition coefficient, K_{ow} values are in the range of 6.5 – 8.0, suggesting a very high potential for bioaccumulation in the organic carbon of sediments and the tendency to reside and accumulate in lipid bilayers, concentrating in aquatic food chains.⁷² Evidence of their widespread bioaccumulation in the environment is documented in many journal articles which have reported the detection of phenolic BZTs in marine organisms in the Ariake Sea, western Japan,⁷⁴⁻⁷⁷ mussels in Asia-Pacific coastal waters,⁷⁸ sediment samples from Pawtuxet River, Narragansett Bay and Rhode Island, USA and Gran Canaria Island, Spain,^{75,76,79-81} wastewater and river water,^{80,81} sludge samples,^{82,83} house dust⁸⁴ as well as

textiles,⁸⁵ reportedly due to incomplete removal in wastewater treatment plants worldwide. Trace amounts of UV-327 was detected recently in human urine samples.⁸⁶

In addition to their persistence in the environment, there are increasing concerns regarding the potential adverse health effects from contact with phenolic BZTs additives incorporated in polymer materials because of the emerging reports on the reproductive and development toxicity in rats,⁸⁷ acute toxicity of UV-571 in fishes⁸⁸ and antiandrogenic activity at high concentrations of exposure.⁸⁹ Japan has prohibited the production and import of UV-320 in 2007. As of December 2015, the category of phenolic BZTs compounds has been nominated for monitoring by the National Toxicology Program (U. S. Department of Health and Human Services)⁹⁰ and is placed in the candidate list of substances of very high concern for authorisation by the European Chemicals Agency (ECHA).⁹¹

1.2.2.3 Metabolic pathways of phenolic benzotriazoles in biological systems

To the best of our knowledge, there is no study on the metabolic pathway of phenolic benzotriazoles in the literature.

1.2.2.4 Literature precedence on possible oxidative metabolites

Identification of the transformation products formed during abiotic or biotic processes (such as hydrolysis, photolysis, oxidation and microbial metabolism) is of great importance especially since the phenolic BZTs are intentionally designed to be stable and some transformation products can be more reactive than the precursor compounds.⁹² However, in contrast to the frequent reports of their occurrence in the environment, there is very limited information in the literature on the oxidative metabolites and their reaction pathways.

The biotransformation pathways of the precursor benzotriazole molecules have been investigated in aquifer materials under aerobic and anaerobic conditions^{93,94} in activated sludge⁹⁵ and biological samples⁹⁶ and the majority of the products formed contain the 5-membered triazole ring. While Liu *et al.*^{93,94} found many products formed from N–N bond scission, Huntscha *et al.*⁹⁵ reported that the aromatic monohydroxylation is the predominant step during the biotransformation of 1H-benzotriazole although alkylation and oxidation are also possible. Asimakopoulos *et al.*⁹⁶ also detected among other products 4-hydroxy-1H-benzotriazole and 5-hydroxy-1H-benzotriazole in human urine samples, showing an unlikely opening of the triazole ring. Benzotriazole has a high aqueous solubility which makes the chemical less likely to accumulate in the environment so attention is switched to the reactions that can occur at the phenolic structure of the phenolic benzotriazoles.

1.2.3 Natural hydroxyl containing compound: Vitamin D

1.2.3.1 *Background of vitamin D and health effects*

Vitamin D refers to a group of naturally occurring secosteroid compounds (similar to steroids but with an open β ring) that exists predominantly in two major forms, namely vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). They can be obtained endogenously in the skin under exposure to sunlight and exogenously from food such as cod liver oil, eggs, breakfast cereals as well as health supplement pills. Vitamin D is a fat soluble vitamin and is the most lipophilic of known antirachitic sterols. Its lipophilicity nature enables vitamin D to be stored and used whenever required by the body.

Deficiency in vitamin D can cause rickets and osteomalacia. An optimal amount of the nutrient is required to regulate blood levels of calcium and phosphorus that is vital for healthy bones. Vitamin D also influences growth and differentiation of many tissues which helps regulate the immune system. Unlike all other vitamins that must be obtained externally from food sources, vitamin D can be synthesized in the skin which led to its classification as a hormone.⁹⁷⁻¹⁰⁰ The key biologically active metabolite of the inert vitamin D, 1,25-dihydroxyvitamin D₃ shares the same cyclopentanoperhydrophenanthrene ring structure as other steroid hormones and reports have shown that it functions in the same way as natural endogenous steroid hormones in homeostasis maintenance.¹⁰⁰

Caution must however be taken regarding the daily intake of vitamin D supplements as an excessive dosage can result in hypercalcemia due to the accumulation of high calcium and phosphorus product levels in the serum. Hypercalcemia can cause soft tissue calcification and stone formation in the urinary tract leading to renal failure, and this dangerous effect of hypervitaminosis D has been observed mostly in infants.¹⁰¹⁻

1.2.3.2 Metabolic pathways of vitamin D in biological systems

Vitamin D is produced mainly in the epidermis of the skin after exposure to UV radiation although small amounts also enter the body through ingestion. Ergosterol and 7-dehydrocholesterol are vitamin D precursors (Scheme 1.4), which react with UV light of wavelengths between 290 – 315 nm to form vitamin D₂ and D₃ respectively. The two compounds are closely related in their structures, with vitamin D₂ containing an additional methyl group and a partially unsaturated phytyl chain.

The vitamin D molecules are biologically inert and must be converted to biologically active forms in order to perform vital roles in calcium homeostasis and bone mineralisation. The metabolic activation of vitamin D occurs *via* two sequential hydroxylation reactions at the carbon-25 of the side chain and then at carbon-1 of the ring. Vitamin D₃ is first metabolized in the liver catalyzed by vitamin D 25-hydroxylase also known as cytochrome P450 2R1 (CYP2R1) oxidative enzymes^{104,105} to form the main circulating form of vitamin D, 25-hydroxyvitamin D₃ (calcifediol) which is then transported to the kidneys and undergoes a second hydroxylation to form 1,25-dihydroxyvitamin D₃ (calcitriol) as shown in Scheme 1.4.⁹⁷⁻¹⁰⁰

Metabolic inactivation of vitamin D occurs *via* multiple step oxidative reactions. 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ are degraded by the catabolic enzyme CYP24A1 which are converted to a series of hydroxylated metabolic products including the less active metabolites 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃ that are eventually oxidized to calcitroic acid, a final excretory product.¹⁰⁶ There has been a study which showed that the 1,25-dihydroxyvitamin D₃ was degraded *via* the phase II glucuronidation pathway.¹⁰⁷

Toxicity effects can arise when the metabolic inactivation pathways are hindered by either the absence of catabolic enzymes responsible for deactivating vitamin D

metabolites or when vitamin D is present in excessive amounts. DeLuca *et al.* proposed that 25-hydroxyvitamin D, among all other active metabolites, is likely the cause of hypercalcemia.¹⁰⁸ The metabolic pathway which leads to vitamin D toxicity remains unclear and there is no consensus on the maximum dose at which vitamin D causes toxicity.

It is believed that vitamin D₂ takes a similar oxidative metabolic pathway as vitamin D₃. The biologically active 1,25-dihydroxyvitamin D (from either vitamin D₂ or D₃) binds to the vitamin D receptor and performs physiological functions in increasing the efficiency of calcium absorption in the intestine.⁹⁷⁻¹⁰⁰ There are however different views on the biological activities of vitamin D₂ and vitamin D₃ even though both undergo similar metabolic transformations. Some studies have reported that vitamin D₃ is considerably 3 ~ 4 times more efficient than vitamin D₂ in maintaining circulating levels of 25-hydroxyvitamin D but there are also studies showing evidence of both being equally effective.¹⁰⁹⁻¹¹³

Scheme 1.4. Metabolic pathway of vitamins D.

1.2.3.3 Literature precedence on possible oxidative metabolites

Chemical oxidation experiments on vitamin D₂ and D₃ have been performed using various methods such as permanganate in ethanol,¹¹⁴ selenium dioxide and hydrogen peroxide in dioxane,¹¹⁵ ozone,¹¹⁶ biomimetic oxidation with a combination of iron-sulfur protein model cluster and oxygen,¹¹⁷ electrolysis using metal salt-hematoporphyrin-O₂-

cathodic reduction system,¹¹⁸ and riboflavin-photosensitized singlet oxygen.¹¹⁹ While the proposed structures of the oxidized products vary, the products showed a common oxidation site at the triene. At least 37 metabolites of vitamin D₃ have been isolated and chemically characterized and are believed to form *via* a series of hydroxylation (activation) and oxidative (inactivation) reactions.¹²⁰

1.3 Investigation of Oxidized Metabolites

1.3.1 *Current analytical methods used for detection of oxidative metabolites*

The metabolites samples used for investigating metabolic pathways are conventionally synthesized in biological *in-vitro* systems such as incubating in animal liver cells microsomal, subcellular S9 fractions or with cytochrome P450 enzymes, or obtained from *in-vivo* specimens such as blood, urine and feces. Incubations are often performed with metabolite trapping agents to stimulate phase II metabolism reactions. Sample preparation methods which include protein precipitation, solid-phase extraction and liquid-liquid extraction remove the complex biological matrices before injecting into the instrument and concentrating the analytes reaching the detector. The major disadvantage of obtaining samples from *in-vitro* and *in-vivo* systems, apart from ethical issues, is the challenging process of purifying the metabolites from the complex matrix. Gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with tandem high resolution mass spectrometry (HRMS) are common techniques used for analyzing and identifying metabolites. They are often complemented with NMR analysis for a definitive assignment of the structural information to the unknowns. Recently, ultra performance liquid chromatography (UPLC) has displayed a higher resolution by separating structurally similar metabolites that would otherwise co-elute in HPLC. These analytical techniques are mostly used to propose a metabolic pathway based on the

identity of the metabolites to establish a correlation between the compound and its ecotoxicological consequence. However, it is not always possible to identify all the metabolites because the techniques depend heavily on the library and not all metabolite standards are available.^{121,122} Other techniques such as capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) have also been successfully applied in the detection of drug metabolites. Their advantage lies in their small reagent and sample consumption as compared to chromatographic techniques.

1.3.2 *Electrochemical methods used for detection of oxidative metabolites*

There have been important advances in linking electrochemical oxidation with UPLC and MS detection to simulate oxidative drug metabolism. In this technique, an oxidation potential is applied to the electrochemical cell to generate reactive intermediates which are separated by UPLC and identified with MS to propose the reaction mechanism of metabolic processes. Studies have reported successful attempts with several pharmaceuticals and their metabolites such as paracetamol,¹²³ sulfur-based drugs,¹²⁴ selegiline,¹²⁵ as well as emerging contaminants such as acebutolol,¹²⁶ triclocarban,¹²⁷ and phenylenediamine¹²⁸ and naturally occurring cytokinins.¹²⁹ The metabolites generated from the electrochemical oxidation were compared with the metabolites generated from either enzyme-catalyzed oxidation or incubation methods and it was found that the products identified were relatively similar.¹³⁰⁻¹³⁸ The exceptions are enantiomeric products. the enzyme-catalyzed oxidations which are often stereoselective, electrochemical oxidation is not enantiospecific so racemic oxidation products are usually obtained.

In addition to oxidation reactions, phase II conjugation reactions can also be simulated by introducing chemical trapping agents like glutathione or cysteine to the

electrochemical cell. Another advantage is that electrochemical oxidation is performed in a clean environment without a complicated biological matrix, hence facilitating the isolation of metabolites for analysis in contrast to biological *in-vitro* systems.¹³⁰⁻¹³⁸

1.4 Rationale and Aim of Thesis

1.4.1 *Using electrochemical methods for studying metabolic reaction mechanisms*

Electron transfer reactions are fundamental processes in enzyme-catalyzed metabolism in biological systems. The metabolic pathways leading to the formation of reactive metabolites are mostly dominated by oxidation processes which are amenable for study by electrochemical methods.¹³⁶⁻¹³⁸ Examination of the oxidative reaction pathways with electrochemical oxidation can therefore be a useful tool for predicting possible oxidation reactions and the identity of reactive intermediates that are relevant to metabolism and toxicity. Furthermore, electrochemical oxidation can also provide valuable insights to finding out the identity of oxidized transformation products that are formed during oxidative water treatments.¹²⁶⁻¹²⁹

Electrochemical oxidation offers several advantages in generating metabolites to be used for structural characterization and establishment of detailed reaction mechanisms. Electrochemical oxidation allows the generation of the metabolites in the absence of a biological matrix which will otherwise require complicated sample extraction procedures. Electrochemical methods are especially useful in examining the exact pathways of redox processes and can be used to generate reactive and short-lived intermediate species as well as determine their stability on the short cyclic voltammetry timescale ($\leq$ seconds) and electrolysis timescale (hours) of long term oxidation products. Electrochemical methods can provide information on the number of electrons involved and the specific

order of the electron transfer steps in the reaction mechanism which are critical in identifying intermediates that are likely to exist in biological systems.

1.4.2 *Performing experiments in aprotic organic solvents*

Bisphenol A, phenolic benzotriazoles and vitamin D compounds are lipophilic in nature which indicates that the three different artificial and natural hydroxyl containing compounds have a higher tendency of residing and accumulating in the hydrophobic lipid bilayer membranes compared to other tissues. Bisphenol A and phenolic benzotriazoles are potential emerging contaminants and the effects of toxicity can be rapid and hazardous considering their ease of entering the bloodstream and accumulating in the body. The high lipid solubility for the two emerging contaminants also translates to a high potential for bioaccumulation in the organic carbon of sediments and concentrating in aquatic food chains. The natural occurring vitamin D exists in hydrophobic cell membranes.

In this thesis, aprotic organic solvents such as acetonitrile, dichloromethane, DMF and DMSO were used as low moisture content solvents because they are closer in properties to the natural lipophilic environment where these compounds are likely to reside and undergo oxidative metabolic reactions. Aprotic organic solvents are also useful media for stabilizing reactive intermediates.¹³⁹

1.4.3 *Aim of thesis*

Although many studies and reviews can be found in the literature on the sources, occurrence and evidence of toxicity effects for the three different types of compounds, their respective metabolic pathways in biological systems are still poorly established. Electrochemical studies on the three artificial and natural hydroxyl containing compounds are also seriously inadequate. There are several reports that have described the

voltammetric behavior of BPA, phenolic benzotriazoles and vitamin D compounds but the majority of these electrochemical studies are performed usually as a means of quantifying the compounds in biological samples or in test solutions that are modeled after physiological environments (such as by chromatography methods with electrochemical detection), rather than for mechanistic studies.

In this thesis, electrochemical studies were performed on the three hydroxyl containing compounds in aprotic organic solvents and at glassy carbon and platinum electrodes. Cyclic voltammetry was performed with varying scan rates and controlled potential electrolysis with coulometry analyses were performed to investigate the number of electrons involved in each oxidation process respectively. The results were complemented with spectroscopic analyses to elucidate and confirm the identities of the electrochemically generated metabolites. The aim of the thesis is to use electrochemical oxidation to mimic oxidative metabolism in order to study the exact redox reaction mechanism of the three compounds in aprotic organic solvents as a model for oxidative reactions that can occur within low water content biological (lipophilic) systems. Gaining a detailed understanding of the mechanistic information involved during the oxidative biotransformation from the parent compound to potentially toxic metabolites as well as the final excreted products will be helpful in examining the potential pathways of chemical conversions that can occur under biological conditions and the investigation of toxicity at the molecular level.

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Chapter 2

Experimental

2.1 *Summary of Electrochemical and Chemical Procedures*

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2.7 *Supporting Electrolyte, Solvents and Chemicals*

References

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2.1 Summary of Electrochemical and Chemical Procedures

In this research, the most frequent technique used for the electrochemical experiments is cyclic voltammetry (CV). Other standard electrochemical procedures used are linear sweep voltammetry (LSV), square-wave voltammetry (SWV), rotating disk electrode (RDE) voltammetry and controlled potential electrolysis with coulometry (CPE) analyses. Depending on the nature of the study in each chapter, the electrochemical experiments were studied together with a combination of spectroscopic analyses such as electron paramagnetic resonance (EPR) spectroscopy, ultraviolet-visible (UV-vis) spectroscopy as well as scanning electron microscopy with energy dispersive x-ray (SEM-EDX). Voltammetric results provide significant clues to the reaction mechanism while information from spectroscopic studies can complement the discussion by confirming the identity of intermediates and final products proposed in the mechanism. High resolution images of the electrode surface were studied with SEM-EDX for examining the morphology of coated electrodes.

Products obtained from chemical oxidation experiments were isolated and characterized by ^1H , ^{13}C and distortionless enhancement by polarization transfer (DEPT) nuclear magnetic resonance (NMR) spectroscopy, high resolution mass spectrometry (HRMS), fourier transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-vis) spectroscopy and x-ray crystallography.

2.2 Theory of Electrochemical Procedures

2.2.1 *Electrochemical cell used in voltammetric experiments*

Figure 2.1 shows a typical electrochemical cell used for standard voltammetric experiments including CV, LSV, SWV and RDE. The electrochemical cell consists of three electrodes (Section 2.3.1), namely the counter or auxiliary electrode (AE), working

electrode (WE), and lastly the reference electrode (RE) which is placed in close proximity to the working electrode to minimize contributions of the ohmic (IR) drop to the applied potential.^{1,2} The IR drop is associated with the uncompensated resistance of the solution and can be minimized by using a supporting electrolyte with a high conductivity. The voltammetric experiments are conducted with the electrodes immersed in an ($\sim 1 - 5$ mM) analyte solution containing a high concentration of (0.2 M) supporting electrolyte (Section 2.7) to suppress ion migration effects and decrease the total resistance of the solution.³⁻⁵

Figure 2.1. Typical electrochemical cell used in voltammetric experiments.

The three electrodes are connected to a potentiostat which regulates the voltage difference between the working electrode and auxiliary electrode when current flows to compensate for majority of the ohmic drop. IR drop occurs across the cell because the solution resists current flow. The auxiliary electrode supplies electrons to counter the oxidation/reduction reaction of the analyte occurring at the working electrode and the

reference electrode provides a constant potential against the potential of the working electrode.²

Low temperature experiments were performed by introducing cool ethanol through the two small glass ports into the jacketed electrochemical cell surrounding the analyte solution (Figure 2.1).

In this research, all electrochemical experiments were conducted with a computer controlled Eco Chemie Autolab PGSTAT302N potentiostat under an argon atmosphere.

2.2.2 Cyclic voltammetry and linear sweep voltammetry

Cyclic voltammetry (CV) is an excellent electroanalytical technique used in the study of electrochemistry and mechanistic understanding of electrochemically active species. Capable of an instantaneous detection of reduction and oxidation (redox) peaks over a wide potential range, CV is frequently used as an important investigative tool in exploring the electrochemistry of new compounds at the molecular level.

In cyclic voltammetry, a potential that changes with time is applied to the working electrode either with the scan starting from an initial potential towards the positive potential direction during the investigation of oxidation processes or with scanning towards the negative potential direction during the investigation of reduction processes. At the start of each CV experiment, the potential is held at the initial potential of zero current flow for a stipulated 5 seconds to equilibrate the system. The triangular waveform illustrated in Figure 2.2a is a typical potential vs. time profile for a single scan CV. The potential at the working electrode is scanned at a fixed rate from an initial potential (E_0) to the switching potential (E_λ) at which the scan direction is reversed and returns to the initial potential for one cycle. The resulting current response is recorded and plotted against the applied potential to give a cyclic voltammogram, as shown in Figure 2.2b.

Linear sweep voltammetry works in the same way as cyclic voltammetry except that the potential is only scanned in one direction from E_0 to E_λ without reversing back to E_0 .⁶

Figure 2.2. (a) Waveform of potential, E as a function of time, t for cyclic voltammetry. (b) A typical cyclic voltammogram of current, i as a function of potential, E obtained for a reversible one-electron oxidation process of **A**.

The cyclic voltammogram shown in Figure 2.2b is typical of a one-electron reversible system in stationary solutions. As the potential is scanned towards values that are sufficiently positive to induce oxidation at the electrode, an exponential increase in the current is observed as a result of the analyte, **A** oxidizing at the electrode surface. **A** is oxidized *via* one electron to form $A^{+\bullet}$ and the depletion of **A** increases with more positive

potentials as the concentration gradient increases. The current continues to increase as the potential approaches the formal potential of **A** until it eventually reaches a maximum (i_p^{ox}) due to concentration polarization because more **A** is required at the electrode surface. More **A** is transported from the bulk solution to replenish the interfacial region and the diffusion layer increases in thickness. At potentials more positive than the oxidative peak potential (E_p^{ox}), the flux of **A** to the electrode decreases and the current decreases as a result, giving a characteristic peak-shaped voltammogram.⁶⁻⁹

During an oxidation process, the electrons are transferred across the interface to the electrode and are conducted away from the solution. The current measured is proportional to the concentration of **A** which is limited by its flux to the electrode surface. When the scan direction is reversed after scanning past the switching potential, $A^{+\bullet}$ is reduced and converted back to the starting material. As shown in Figure 2.2b, the oxidative peak current has a similar size as the reductive peak current which is indicative of a chemically reversible process ($i_p^{red}/i_p^{ox} = 1$) where i_p^{red} is the reductive peak current and i_p^{ox} is the oxidative peak current.

A clear distinction however, must be made between the terms electrochemical reversibility and chemical reversibility. The electrochemical reversibility relates to the rates of the heterogeneous electron transfer while chemical reversibility relates to the lifetimes of the oxidized and reduced species involved in the electron transfer step.¹¹ Electrochemical reversibility is usually determined by examining the peak-to-peak separation (ΔE_{pp}) between the oxidative peak potential (E_p^{ox}) and the reductive peak potential (E_p^{red}) while chemical reversibility is determined by examining the i_p^{red}/i_p^{ox} ratios. In this research, the terms chemical reversibility and chemical irreversibility are often used and it should be noted that the terms used in the context of cyclic voltammetry refer to the stability of the oxidized species in the CV timescales $\leq$ a few seconds, and do

not refer to the electrochemical reversibility with respect to the heterogeneous electron transfer step.

2.2.2.1 Chemical reactions coupled to electron transfer processes

Homogeneous chemical reactions are often coupled to the heterogeneous electron transfer steps.¹⁰⁻¹² By performing consecutive scans over the potential range and varying the voltammetric scan rates using cyclic voltammetry, it is possible to construct a reaction mechanism from the information obtained regarding the lifetimes of the oxidized and reduced species and their probable follow up homogeneous chemical reactions or heterogeneous electron transfer steps. For example, the oxidation mechanism for phenols including the well-studied example of 2,4,6-tri-*tert*-butylphenol¹³⁻¹⁶ is generally known to occur in three steps *via* an ECE mechanism beginning with an initial $1e^-$ oxidation to form the phenol cation radical (E step), followed by a deprotonation to form a neutral radical (C step) and a further one electron oxidation to a phenoxonium cation (E step) as shown in Scheme 2.1.

Scheme 2.1. General mechanism for electrochemical oxidation of phenols.

2.2.3 Square-wave voltammetry

Square-wave voltammetry (SWV) is a pulse voltammetric technique commonly used in electroanalytical quantitative studies because of the advantage of a lower limit of detection than CV and LSV. As the SWV measurements are obtained from the net

difference between forward and reverse currents, the background capacitance current is reduced to a minimal, giving an excellent sensitivity.

The excitation signal in square-wave voltammetry is a superimposition of a regular square-wave onto an underlying staircase. During each square-wave cycle, the current is measured twice which is indicated by the solid and shaded dots in Figure 2.3. In each cycle, the first measurement (solid dot) is taken at the end of the forward pulse and the second measurement (shaded dot) is taken at the end of the reverse pulse. The difference between the two current values, Δi is computed and plotted against the applied potential, E to give a peak-shaped voltammogram.¹⁷⁻²⁰ The difference in the current, Δi is directly proportional to the concentration of the electroactive species and the potential of the peak corresponds to the half-wave potential, $E_{1/2} = (E_p^{\text{ox}} + E_p^{\text{red}})/2$ of the oxidized or reduced species. This allows a clear detection of the voltammetric oxidative and reductive peaks as well as the measurement of the half-wave potential even at low analyte concentrations of $10^{-4} - 10^{-5}$ mM.

Figure 2.3. Waveform of potential, E as a function of time, t for square-wave voltammetry (bold lines). The potential applied to the working electrode is a square-wave pulse of amplitude, ΔE_s superimposed on a staircase waveform of pulse height, ΔE_p .

2.2.4 *Rotating disk electrode voltammetry*

By introducing forced convection such as stirring, a sigmoidal shaped voltammogram is obtained instead as illustrated in Figure 2.4. The rotating disk electrode (RDE) technique is a hydrodynamic voltammetric method used to study reactions under steady state conditions. Unlike cyclic voltammetry that is conducted in transient conditions, the reactant is now transported from the bulk solution to the electrode surface by a combination of diffusion and convection. The solution is effectively divided into a stagnant diffusion layer (where purely diffusional transport takes place from the edge of the Nernst diffusion layer to the electrode surface) immediately adjacent to the electrode surface and a well stirred bulk region (where convection takes place from the bulk solution to the edge of the Nernst diffusion layer).

As the rotating disk electrode rotates, it pulls the solution towards itself, spins and then releases in a radial direction, setting up a laminar vortex flow. A steady state is attained with a constant diffusion layer thickness such that fresh starting material is continuously replenished at the electrode surface and the electrolyzed product transported back to the bulk solution. This is observed in Figure 2.4 where the RDE voltammetric scan is maintained at the limiting current. The convection mass transport can be controlled by varying the rotation rate. As the rotation rate is increased, the thickness of the diffusion layer decreases and the flux to the electrode increases, giving rise to a larger limiting current.²¹⁻²³ RDE voltammetry is useful in the determination of the number of electrons transferred during the redox process as well as calculating the diffusion coefficient of the analyte molecules using the Levich equation.

The Levich equation^{23,24} predicts that the current is proportional to the square root of the angular rotation speed (ω) and the concentration of the analyte (C_0^*):

$$i_l = 0.62 n F A D_0^{2/3} \omega^{1/2} \nu^{-1/6} C_0^* \quad (2.1)$$

where i_l is the mass transport limiting current, n is number of electrons transferred, F is the Faraday's constant (96485 C mol^{-1}), A is the area of electrode in cm^2 , D_0 is the diffusion coefficient in $\text{cm}^2 \text{ s}^{-1}$, ω is the angular rotation rate in radians s^{-1} , ν is the kinematic viscosity in $\text{cm}^2 \text{ s}^{-1}$ and C_0^* is the concentration of the analyte in mol cm^{-3} .

RDE experiments were conducted using a Metrohm Autolab RDE-2 rotator with 3 mm diameter planar GC and Pt electrodes.

Figure 2.4. Linear sweep voltammogram recorded at a rotating disk electrode.

2.2.5 Controlled potential electrolysis and coulometry

Controlled potential electrolysis (CPE) experiments are performed with coulometry analyses for an accurate determination of the number of electrons transferred during the oxidation/reduction process and also to estimate the lifetime of the oxidized/reduced species, which is not possible from the CV data alone. CV experiments usually give an indication of the stability of the oxidized species in a short time frame (in

seconds) whereas the timescale of the bulk electrolysis experiments can indicate if they are stable over longer time intervals.

For a bulk oxidative electrolysis, a constant potential that is approximately 100 mV – 200 mV more positive than the E_p^{ox} -value obtained by the CV measurements was applied to the working electrode to ensure that a complete oxidation was achieved. A known concentration of the analyte solution was added to the working electrode compartment of the electrolysis cell and the number of electrons transferred during the electrolysis process (n) is calculated from^{25,26}

$$Q = \int_0^t i \, dt \quad (2.2)$$

$$N = Q/nF \quad (2.3)$$

where i is current in amperes, t is the duration of electrolysis in seconds, Q is charge in coulombs, N is the no. of moles of starting material and F is the Faraday constant (96485 C mol⁻¹). The current is integrated by the computer controlled Eco Chemie Autolab General Purpose Electrochemical System (GPES) program in equation 2.2 to give the area under the i vs. t graph (Q , quantity of electrical charge passed) which is then converted into the number of electrons by substituting into equation 2.3.

As shown in Figure 2.5, the controlled potential electrolysis experiments were performed in a two-compartment electrolysis cell separated with a porosity no. 5 (1.0 – 1.7 μ m) sintered glass frit.²⁷ The porous frit allows adequate current to be passed between the two electrodes. Large electrodes were used for electrolysis to provide a sufficiently large surface area for exhaustive reaction to take place over a reasonable time frame. In Chapter 3, a GC rod was used as the working electrode and a Pt mesh plate was used as the auxiliary electrode. In Chapter 4, the working and auxiliary electrodes used were two identically sized GC cylinders. In Chapter 5, the working and auxiliary electrodes used

were two identically sized Pt mesh plates. In all CPE cells, both working and auxiliary electrodes were symmetrically arranged with respect to each other with an Ag wire reference electrode (isolated from the working electrode compartment by a salt bridge) positioned to within 2 mm of the surface of the working electrode. A 1 mm GC working electrode was also positioned in the working electrode compartment to be used for the recording of the CV before and immediately after the completion of electrolysis. Both compartments were purged constantly with argon gas during electrolysis in order to increase mass transfer to the electrode surface for an efficient electrolysis.

Figure 2.5. Two-compartment electrolysis cell used for controlled potential electrolysis.

2.3 Electrodes

2.3.1 *Standard electrodes*

The working electrode is a 1 mm diameter planar glassy carbon (GC) disk or platinum (Pt) disk (Cypress Systems) used in conjunction with a platinum wire (Pt) auxiliary electrode (Metrohm) and a silver (Ag) wire miniature reference electrode (Cypress Systems) connected to the test solution *via* a salt bridge containing 0.5 M *n*-Bu₄NPF₆ in CH₃CN. Working electrodes used during RDE experiments are 3 mm diameter planar Pt or GC electrodes. Prior to each electrochemical scan, the working electrode was cleaned by polishing with an alumina oxide slurry (grain size 0.3 μm) on a Buehler Ultra-pad polishing cloth, rinsing with ultrapure water, then acetone and dried.

Ferrocene (Fc) is commonly used as an internal standard in non-aqueous solvents as it displays ideal electrochemical reversible behavior *via* a one electron transfer process. Accurate potentials were obtained using ferrocene that was added to the test solution at the end of the measurements so as to minimise problems associated with liquid junction potentials and to provide an internal standard when different reference electrodes were used in the measurements. Ferrocene is oxidized at approximately +0.64 V *vs.* SHE (standard hydrogen electrode) in CH₃CN.²⁸

2.3.2 *Electrode fabrication*

The working electrodes used in Chapter 6 are copper wires with a diameter of 1 mm (99.99+%) from Goodfellow. Copper electrodes were prepared by sealing the wires inside heat-shrink Teflon tubes with Araldite resin/hardener (Figure 2.6a). After curing, the end of the tubes was sawn off and the surface was mechanically polished with a set of abrasive papers in the following order: 400, 1200, 2000 and 4000 grit (Metrohm EasyCon). Multi-walled carbon nanotubes (MWCNTs) were applied to the electrodes by

repeatedly coating the electrode with 0.5 μL droplets of the MWCNTs suspended in DMF. Each droplet was allowed to dry before the next application (Figure 2.6b). The working electrode was used in conjunction with a platinum wire (Pt) auxiliary electrode (Metrohm) and a Ag/AgCl (3 M KCl) reference electrode (Metrohm). A new copper electrode surface was prepared freshly for every electrochemical scan. A height of approximately 2 mm of the copper electrode was sawn off and polished to achieve a fresh surface after each scan.

Figure 2.6. (a) Two 1 mm Cu wires sealed in Araldite resin/hardener. (b) 1 mm Cu electrode prepared after curing and mechanical polishing.

2.4 Controlled Conditions Experiments

2.4.1 *Low temperature*

Electrochemically generated intermediate species can be extremely reactive and unstable so experiments were performed at low temperatures in attempts to increase their lifetimes. Variable temperature (–40 °C to 20 °C) cyclic voltammetric experiments were performed in a Metrohm jacketed glass cell with the temperature controlled using a Julabo FP89-HL ultralow refrigerated circulator that uses ethanol as the circulating bath solution.

2.4.2 *Light sensitive*

In Chapter 4 and Chapter 5 respectively, phenolic benzotriazoles and vitamin D compounds are light sensitive. The compounds are stored in the dark and the experiments are prepared and performed under dim lights to prevent photodecomposition.

2.5 Chemical Oxidation Experiments

Chemical oxidation experiments were conducted by adding a weighed quantity of nitrosonium hexafluoroantimonate (NOSbF₆) in the desired stoichiometric amount to a solution containing the starting material and stirring the mixture at room temperature for approximately 30 min. NOSbF₆ (99.9%) used for chemical oxidation experiments was obtained from Sigma-Aldrich and stored in a glovebox in a nitrogen atmosphere.

2.6 Spectroscopy and Microscopy Techniques

2.6.1 *Electron paramagnetic resonance spectroscopy*

Electron paramagnetic resonance (EPR) spectroscopy, also known as electron spin resonance (ESR), is especially useful in detecting and identifying electrochemically generated intermediates and products that contain unpaired electrons such as free radicals and radical ions.²⁹⁻³¹ Due to the Zeeman effect, the energy levels of an unpaired electron are split by an external magnetic field into two spin states, m_s of $+\frac{1}{2}$ and $-\frac{1}{2}$ as shown in Figure 2.7a. The EPR spectrum is recorded by measuring the absorption by the paramagnetic samples while sweeping the magnetic field (Figure 2.7b). An additional hyperfine splitting can occur in paramagnetic samples that contained magnetic nuclei with a non-zero nuclear spin such as equivalent neighbouring protons or other nuclei (^{14}N and ^{31}P) due to the interaction between the magnetic moments of the unpaired electron and the magnetic nuclei.

Information obtained from the hyperfine splitting patterns is used to characterize the environment of the paramagnetic species and deduce the structure. EPR spectra can also be used to determine the electron transfer rates of the reaction as the spectral line widths vary with effects of concentration.³¹ In this research, we have used EPR to determine the concentration of the paramagnetic species generated from electrolysis. The derivative EPR spectrum was converted into an absorption spectrum by two integrations and compared quantitatively with a radical standard of known concentration.

Solution-phase EPR spectra were recorded in a silica flat cell with a Bruker Biospin ELEXSYS-II E500 EPR spectrometer operating in continuous-wave X-band mode with the following instrumental settings: number of accumulations = 10 scans, modulation amplitude = 1 G, attenuation = 25 dB, sweep time was 30.72 s and the time constant was 10.24 ms. All sample solutions (1 mM) were electrolyzed according to the

controlled potential electrolysis procedure described in Section 2.2.5 before being transferred immediately to a quartz flat cell for EPR spectroscopy analysis. All EPR spectroscopic experiments were conducted at 22 ± 2 °C.

Figure 2.7. (a) Energy-level diagram of a free electron in a magnetic field. (b) EPR spectrum of absorption vs. magnetic field. (c) Derivative EPR signal.

2.6.2 *Ultraviolet-visible spectroscopy*

Ultraviolet-visible (UV-vis) absorption spectroscopy provides good supplementary information in the proposal of a reaction mechanism as strong absorbance bands of the electrochemically generated intermediates or products can be detected instantaneously. UV-vis spectra were recorded on a Perkin-Elmer Model Lambda 750 UV-vis NIR spectrophotometer. All sample solutions (1 mM) were electrolyzed according to the controlled potential electrolysis procedure described in Section 2.2.5 before being transferred immediately to a cuvet for UV-vis spectroscopy analysis. All UV-vis experiments were conducted at 22 ± 2 °C.

2.6.3 *Scanning electron microscopy with energy dispersive x-ray*

Scanning electron microscopy with energy dispersive x-ray (SEM-EDX) spectroscopy is a widely used technique for analysing surfaces in a vacuum environment. SEM provides detailed high resolution images *via* the detection of secondary electrons that are emitted from the specimen surface after irradiated with an electron beam. EDX is used for the identification of the elements found on the specimen surface as well as elemental mapping to show the relative proportions in atomic %. Overall, SEM-EDX is a very powerful technique used for the determination of the morphology and chemical composition of surfaces.³²

In Chapter 6, morphological analysis of bare Cu and MWCNT-modified Cu electrodes were performed using a JEOL 7600F field-emission SEM with an acceleration voltage of 2 and 5 kV. Copper electrode samples were adhered on an aluminium sample holder using conductive silver paint. A maximum of 5 kV was used as the acceleration voltage to achieve the objective of depth profiling and to detect the majority of the lighter elements.

2.7 Supporting Electrolyte, Solvents and Chemicals

The supporting electrolyte tetra-*n*-butylammonium hexafluorophosphate (*n*-Bu₄NPF₆) was prepared according to a literature procedure³³ by reacting equal molar amounts of 40% aqueous solution of *n*-Bu₄NOH with 65% aqueous solution of HPF₆, washing the precipitate with water and recrystallising thrice from hot ethanol followed by drying under vacuum at 160 °C for 24 h and then storing under vacuum. Tetra-*n*-butylammonium hydroxide (*n*-Bu₄NOH, 40% in water) was obtained from Alfa Aesar and hexafluorophosphoric acid (HPF₆, 65% in water) was obtained from Fluka.

The solvents used throughout the project were of analytical grade and used directly from the bottles. Acetonitrile (CH₃CN) was purchased from Merck, ACI labscan and Anhui Fulltime Solvents & Reagents Co., Ltd. Dichloromethane (CH₂Cl₂), N,N-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were obtained from Tedia. Ultrapure water with a resistivity of 18.2 MΩ.cm was obtained freshly from an ELGA Purelab Option-Q water purification system.

In Chapter 3, BPA (≥ 99%) was obtained from Sigma-Aldrich. Trifluoroacetic acid, CF₃COOH (>99%) was obtained from Tokyo Chemical Industry (TCI).

In Chapter 4, UV-234 (CAS#: 70321-86-7) 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (> 98%) and UV-327 (CAS#: 3864-99-1) 2,4-di-tert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol (> 98%) were obtained from Tee Hai Chem and stored in the dark at room temperature. The base used for deprotonation of phenols was *n*-Bu₄NOH which was also used in the synthesis of the electrolyte. Tetracyanoquinodimethane (TCNQ) was obtained from Sigma-Aldrich.

In Chapter 5, vitamin D₂ (≥ 98%) and vitamin D₃ (≥ 97%) were obtained from Sigma-Aldrich and stored in the dark at 4 °C.

In Chapter 6, multi-walled carbon nanotubes (MWCNTs, > 95%, 50 – 80 nm

diameter, 10 – 20 μm length) were synthesized by catalytic chemical vapor deposition (CVD) and were obtained from Nanostructured & Amorphous Materials Inc. (Houston, Texas). MWCNTs were dispersed in DMF (1 mg in 1 mL) and sonicated in an ultrasonic bath for 15 min after which an aliquot of 0.5 μL was cast onto the electrode. The suspension was allowed to evaporate by placing the electrode under a stream of N_2 gas. 0.1 M sodium sulfate (Na_2SO_4) solution was prepared in ultrapure water from analytical grade Na_2SO_4 obtained from Schedelco. Potassium ferricyanide and potassium ferrocyanide were obtained from Merck. Aluminium oxide powder (0.3 μm grain size) was obtained from Metrohm. The alumina powder was dispersed in DMF (2 mg in 1 mL) by sonicating in an ultrasonic bath for 15 min after which an aliquot of 0.5 μL was cast onto the electrode.

Unless otherwise specified, all chemicals and reagents were of analytical grade and used as received.

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Chapter 3

Electrochemical/Chemical Oxidation of Bisphenol A

3.1 *Literature Review of Electrochemical Studies Performed on Oxidation of Bisphenol A*

3.2 *Results and Discussion*

3.2.1 *Cyclic voltammetry and adsorption effects from oxidation of bisphenol A*

3.2.2 *Controlled potential electrolysis*

3.2.3 *Chemical oxidation*

3.2.4 *General mechanism for electrochemical oxidation of phenols*

3.2.5 *Proposal of reaction mechanism for the oxidation of bisphenol A*

3.3 *Conclusions*

References

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3.1 Literature Review of Electrochemical Studies Performed on Oxidation of Bisphenol A

The electrochemical behavior of bisphenol A (BPA) has been extensively studied in aqueous buffered solutions.¹⁻²² Electrochemical studies were mostly conducted in aqueous solutions with the aim of exploring electrochemical oxidation methods to treat water contaminated with BPA¹⁻⁹ or to chemically modify electrodes for a fast and sensitive detection of BPA in the environment.¹⁰⁻²² A large number of studies were performed at a wide range of modified electrodes incorporating nanomaterials such as carbon nanotubes (CNTs),¹⁰⁻¹² carbon nanohorns (CNHs),¹³ quantum dots,¹⁴ chitosan-Fe₃O₄ nanocomposite¹⁵ and gold nanoparticles,¹⁶ as well as reactions mediated by ruthenium (II) complexes at an indium tin oxide (ITO) electrode¹⁷ and carbon electrodes modified with stacked graphene nanofibers/gold nanoparticle composites,¹⁸ Mg-Al-CO₃ layered double hydroxides,¹⁹ fullerenes,⁹ non-covalently modified ionic liquid-based β -cyclodextrine,²⁰ cobalt phthalocyanine²¹ and tyrosinase enzymes²² in aqueous buffered solutions.

Electrochemical studies of BPA oxidation performed at unmodified electrodes such as platinum,⁷ glassy carbon²³ and boron-doped diamond²⁴ reportedly show poor selectivity and sensitivity in their responses due to a fouling effect at the unmodified metallic surfaces. It is widely reported that an electrode modification can potentially decrease the oxidation overpotential and improve the stability of the electrode and current responses.¹⁰⁻²² However, a fouling phenomenon – adsorption of polymeric films of BPA was often still observed on the modified electrode surfaces in aqueous solutions.^{4,15,17,19,21} The adsorption processes that occur during electrochemical oxidation result in diminishing oxidative peak currents (i_p^{ox}) with repetitive scanning. It was proposed that the polymeric film was formed due to electrochemical polymerization. BPA could be

electrochemically oxidized *via* a $-2e^-/-2H^+$ process to produce phenoxyl radicals which first combined to form dimers and then are further oxidized to new diradicals that couple randomly among themselves to form polymers that vary in structure (Scheme 3.1, equation 3.1).^{4,6,8,14,15,19} For the water soluble products, it was suggested that BPA was oxidized *via* a two-electron and two-proton process^{4,10-14,16,19-21} and the oxidized product was proposed to be bisdienone, whereby the two phenolic groups on BPA were oxidized to carbonyl groups (Scheme 3.1, equation 3.2).^{10-13,20,21} There is also a study by Niu *et al.*¹⁸ who reported a four-electron and four-proton process and proposed the formation of dihydroxybenzene at each phenolic group on BPA, which was then oxidized to four carbonyl groups (Scheme 3.1, equation 3.3). The identities of some oxidized products of BPA and their oxidative reaction pathways were proposed in the literature but the structure and the number of electrons and protons involved in the proposed mechanisms vary.

As far as we are aware, there are very few reports providing mechanistic information on the electrochemical oxidation of BPA in aprotic organic solvents. In aqueous systems, hydroxyl radicals can be generated at the electrode surface, which then proceed to bring about BPA degradation. In organic solvents, electrochemical oxidation does not generate hydroxyl radicals and hence, it is possible that BPA and its potential metabolites react differently in organic and aqueous solvents.

In this chapter, we examine the electrochemical oxidation mechanism of BPA in order to investigate its redox reaction mechanisms in aprotic organic solvents as a model for oxidative reactions that can occur within low water content biological (lipophilic) systems as well as to identify whether the reaction mechanism is similar to that observed for other phenols.

Scheme 3.1. Speculative oxidation products of BPA reported in the literature.^{4,8,14,18,20}

3.2 Results and Discussion

3.2.1 Cyclic voltammetry and adsorption effects from oxidation of bisphenol A

Previous electrochemical studies on BPA in water showed an oxidative process on the forward scan and no reductive peak when the scan direction was reversed.^{4,7,9-14,16,17,19-21,23,24} Because adsorption effects can impede the study drastically, the electrochemical behavior of BPA was first tested in four aprotic organic solvents namely CH₃CN, CH₂Cl₂, DMF and DMSO and a mixture of 80% CH₃CN: 20% H₂O solution to determine an optimal solvent composition to work with. The cyclic voltammograms obtained for BPA in all solvents tested showed an anodic oxidation process(es) with oxidation peak

potentials (E_p^{ox}) between +0.60 V to +1.00 V vs. Fc/Fc^+ , with no evidence of a reverse cathodic (E_p^{red}) peak (Figure 3.1). Thus, it can be concluded that in organic solvents BPA undergoes a chemically irreversible voltammetric oxidation process to form compounds that cannot be electrochemically converted back to the starting materials (at least on the CV timescale of $\leq$ a few seconds), which is similar to the results obtained in water. The intermediate oxidized form of BPA is chemically short lived, likely undergoing a follow up homogenous chemical reaction(s) after the initial heterogeneous electron transfer oxidation step(s).

The appearance of the voltammograms obtained for BPA in DMF and DMSO were somewhat different from those obtained in CH_3CN and CH_2Cl_2 solutions (Figure 3.1). CVs obtained for BPA in DMF and DMSO solutions showed two closely spaced oxidative peaks during the forward oxidative process whereas the latter showed one oxidative peak, although the CVs obtained in CH_2Cl_2 displayed a particularly broad peak, suggesting overlapping voltammetric processes. Since the voltammetric process(es) appear chemically irreversible (show no reverse peak when the scan direction is reversed) in all solvents, the second process that is detected at more positive potentials in DMSO and DMF is associated with the oxidation of a product of the initial electron(s) transfer/homogenous chemical reaction. It is possible that these new oxidized products of BPA are themselves oxidized at different potentials in DMSO and DMF than observed in CH_3CN or CH_2Cl_2 , due to specific solvent-substrate interactions, therefore, are not observable within the same potential range. An alternative explanation is that the oxidation pathway does not go by exactly the same route in DMF/DMSO compared to in CH_3CN or CH_2Cl_2 , so different reaction products are produced. The oxidative peak currents (i_p^{ox}) values for the oxidation process were also much lower in DMF and DMSO compared to those recorded in CH_3CN and CH_2Cl_2 (Figure 3.1). The lower peak currents

can partly be explained by the smaller diffusion coefficient values in the more viscous solvents DMF and DMSO. A further observation was that BPA is more easily oxidized at less positive potentials in DMF and DMSO compared to in CH_3CN and CH_2Cl_2 .

Adsorption phenomena were observed during the CV experiments in the organic media such that the i_p^{ox} values decreased noticeably during the second scans in all solvents tested (Figure 3.1a). The decrease in the i_p^{ox} for the second scan however, became less pronounced in Figure 3.1b when the potentials were cycled to more negative potentials, and it appears that the adsorbed species can be reduced and at least partially stripped off the electrode by applying sufficiently negative potentials. A different negative potential limit was used for each solvent because of their different limits of stability. Each solvent has a different potential window in which voltammetric studies can be conducted within and scanning past the negative potential limit will result in a steep decrease in current due to the solvent undergoing reduction at the electrode.

Figure 3.1. Cyclic voltammograms of two consecutive scans of 2 mM BPA recorded at a scan rate of 0.1 V s⁻¹ in a range of solutions with 0.2 M *n*-Bu₄NPF₆ at a 1 mm GC electrode at 22 ± 2 °C over (a) shorter potential range, and (b) longer potential range. (—) First scan. (···) Second scan.

3.2.2 *Controlled potential electrolysis*

The oxidation of BPA showed the least adsorption effects in DMF and DMSO as elucidated from the shapes of the voltammograms, with the second scan more closely overlapping the first scan compared to CH₃CN and CH₂Cl₂ (Figure 3.1). Therefore, to minimize adsorption effects, DMSO was chosen as the solvent to perform controlled potential electrolysis experiments, and also because BPA oxidizes more easily in DMSO and so a complete bulk oxidation may be achieved more readily.

Bulk controlled potential electrolysis and coulometry experiments were performed on BPA. The potential applied during the CPE experiments was +0.80 V vs. Fc/Fc⁺, which was approximately 150 mV more positive than the first E_p^{ox} -value obtained by CV experiments in DMSO. The applied potential was theoretically sufficiently positive to bring about the complete oxidation of the analyte. To overcome the problem of electrode fouling from the adsorption of oxidized products of BPA on the electrode surface as the electrolysis proceeded, the applied potential was switched periodically to a negative potential in between oxidative electrolysis runs to strip adsorbed species off the electrode. This additional step termed pulsed stripping was performed at a potential of -1.70 V vs. Fc/Fc⁺ for approximately 30 – 60 s when the electrolysis current was observed to drop below 1 mA at the large Pt mesh working electrode (which was used as a gauge that the electrode had fouled; minimal current was passing and further bulk oxidation was extremely slow).

Cyclic voltammograms performed at the beginning and after electrolysis (incorporating pulsed stripping by alternating several cycles of oxidative electrolysis and pulsed stripping steps) which took over 3 h at 22 ± 2 °C are shown in Figure 3.2. While performing pulsed stripping at a negative potential of -1.70 V vs. Fc/Fc⁺ seemed to be effective in regenerating the electrode for the short time-scale cyclic CV experiments

(obtained over several seconds) as illustrated in Figure 3.1, it did not however work for exhaustive long time-scale controlled potential oxidative electrolysis (obtained over minutes to hours). The CV obtained at the end of the electrolysis [Figure 3.2a (red line)] showed no obvious change from that of the starting material [Figure 3.2a (black line)] even though a transfer of 0.36 electrons (n value) per molecule was recorded. The reason is likely due to a larger amount of the oxidized product adsorbing on the working electrode surface during electrolysis, which makes it more difficult to strip off by applying a negative potential.

Previous studies by Murugananthan *et al.*¹ indicated that it was possible to remove the fouling layer on the electrode by anodic polarization at the potential region of water decomposition > 2.30 V *vs.* saturated calomel electrode (SCE). It was suggested that the adsorbed polymeric film can be oxidized into smaller un-adsorbed molecules by hydroxyl radicals generated during decomposition of water. It was not possible to study the electrochemical behavior of BPA at potential greater than $+1.00$ V *vs.* Fc/Fc^+ during cyclic voltammetric experiments because the solvent begins to undergo oxidation at that potential. DMSO has a small solvent window of approximately -3.00 V to $+1.00$ V *vs.* Fc/Fc^+ . The CPE experiment was nevertheless repeated with the pulsed stripping performed at $+1.30$ V *vs.* Fc/Fc^+ but the attempt to oxidize BPA completely was also not successful despite performing the electrolysis over a duration of more than 3 h. The CV obtained at the end of the electrolysis [Figure 3.2b (red line)] showed little change from that of the starting material [Figure 3.2b (black line)]. It is likely that the bulk of the electrons transferred (apparent $n \sim 0.93$) comes from the solvent being oxidized at the positive potentials and any reaction that converts the adsorbed species to un-adsorbed forms is unlikely. The adsorption effects were clearly blocking the electrodes and further electrolysis was not possible.

Figure 3.2. Cyclic voltammograms recorded at a scan rate of 0.1 V s^{-1} with a 1 mm diameter GC electrode during controlled potential electrolysis of 5 mM BPA in DMSO with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ at $22 \pm 2^\circ\text{C}$. The CPE experiment was performed by incorporating a pulsed stripping step in between oxidative electrolysis runs in attempts to overcome electrode fouling. (a) Pulsed stripping at $-1.7 \text{ V vs. Fc/Fc}^+$ for 30 – 60 s. (b) Pulsed stripping at $+1.3 \text{ V vs. Fc/Fc}^+$ for up to 900 s. n is the apparent number of electrons transferred during electrolysis; (black line) before the bulk oxidation of BPA, (red line) after bulk oxidation conducted for over 3 h.

3.2.3 Chemical oxidation

A fast and straightforward method for oxidizing phenols was previously achieved with the use of NOSbF_6 as a one-electron chemical oxidant, which closely mimicked the results of electrochemical oxidation experiments.²⁵ The procedure simply involves adding a weighed quantity of NOSbF_6 in the desired stoichiometric amount to a solution

containing the phenolic compound and stirring the mixture at room temperature for approximately 30 min. Chemical oxidation experiments were conducted on BPA because it was not possible to use electrolysis due to electrode fouling from the adsorption effects. Another advantage of using chemical oxidation is that it occurs considerably faster than electrochemical oxidation and avoids the use of the supporting electrolyte which can be difficult to remove when trying to isolate the oxidized products. Furthermore in the case of NOSbF_6 , the byproduct, $\text{NO}^\bullet$ (reduced form of NO^+) exists as a gaseous product and can be removed easily from the solution.²⁶ Chemical oxidation experiments were performed on BPA using NOSbF_6 in the solvent CH_3CN , since it has a much lower boiling point than DMSO and is easier to remove during the product work-up stages.

The chemical oxidation of BPA with varying molar equivalents of NO^+ was first analyzed in conjunction with cyclic voltammetry, in order to determine the equivalents of NO^+ required to achieve complete oxidation, and hence to calculate the number of electrons transferred during the process. It was found that the main oxidation peak of BPA at $E_p^{\text{ox}} = +1.00 \text{ V vs. Fc/Fc}^+$ shifted and reduced in size after the addition of the 1st and 2nd mol equiv. of NO^+ respectively (Figure 3.3), but the exact changes in the shapes of the voltammograms are difficult to explain due to the complicated homogeneous reactions (likely proton transfers) that are also occurring following the electron transfer steps. The wave belonging to the starting material disappears completely and a new product is formed at a more positive potential at $+1.30 \text{ V vs. Fc/Fc}^+$ after the addition of 4 mol equiv. of NO^+ , indicating the transfer of 4 electrons for exhaustive oxidation. The recorded voltammogram of the product remained similar with the addition of a 5th equivalent and it was hence confirmed that a total of 4 electrons were involved in the oxidation of BPA. Some unknown compounds, likely a mixture of products were also formed concomitantly to the addition of NO^+ and were detected voltammetrically at the

negative potentials (~ -1.00 V vs. Fc/Fc^+), which increased proportionally with increasing additions of the oxidant up until the 4th equivalent (Figure 3.3).

Figure 3.3. Cyclic voltammograms of 2 mM BPA with the addition of 1 – 5 molar equivalents of NOSbF_6 (one-electron chemical oxidant) recorded at a scan rate of 0.1 V s^{-1} in CH_3CN with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ at a 1 mm GC electrode at $22 \pm 2^\circ\text{C}$.

The reductive wave observed at ~ -1.00 V vs. Fc/Fc^+ did not increase in size when the 5th equivalent of NO^+ was added, which indicates that the wave was most likely due to intermediate species or products formed during the oxidation of BPA with 4 equivalents of NO^+ . The possibility of the wave belonging to H^+ released during oxidation was ruled out *via* the standard addition of acid. The mechanism for the oxidation of BPA, which is discussed in detail in Section 3.2.5, was proposed to involve the release of 4H^+ ions. The cyclic voltammogram of 8 mM trifluoroacetic acid, CF_3COOH (4 equivalents) was

recorded in acetonitrile solution to test the CV response for H^+ ions (Figure 3.4), but the addition of acid did not lead to a reductive peak at ~ -1.00 V vs. Fc/Fc^+ . It is also unlikely that the wave was related to the NO^+ (or $\text{NO}^\bullet$) present in excess since the reductive process at -1.00 V vs. Fc/Fc^+ shown in Figure 3.3 did not increase in size as more NO^+ were added after the reaction was complete (after the addition of 4 equivalents of NO^+). The cyclic voltammogram of 8 mM NOSbF_6 (4 equivalents) however, showed a reduction peak at ~ -2.00 V vs. Fc/Fc^+ which suggested the presence of some $\text{NO}^+/\text{NO}^\bullet$ in solution.

Figure 3.4. Cyclic voltammograms of 8 mM trifluoroacetic acid (4 equiv.), 8 mM NOSbF_6 (4 equiv.) and the reaction mixture of 8 mM NOSbF_6 added to 2 mM BPA solution recorded respectively at a scan rate of 0.1 V s^{-1} in CH_3CN with $0.2 \text{ M } n\text{-Bu}_4\text{NPF}_6$ at a 1 mm GC electrode at $22 \pm 2^\circ\text{C}$.

Synthetic scale oxidation experiments were conducted by reacting 0.175 M of BPA with 4 mol equiv. of NOSbF_6 in CH_3CN . NOSbF_6 (4 equiv) was added to a solution of BPA (200 mg, 0.175 mol) in 5 mL of CH_3CN at $22 \pm 2^\circ\text{C}$. The solution became black immediately and was stirred for another 15 min before checking with thin layer

chromatography (TLC) for complete reaction. The solvent was then removed under vacuum and the residue was separated by column chromatography and then preparative thin layer chromatography to give a brown solid (**1**), 4,4'-(propane-2,2-diyl)diphenol (20 mg, 9%). mp 101 – 102 °C. IR: 3283, 1659, 1603, 1582 cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ : 7.91 (d, 4H, $J = 8.8$ Hz), 7.17 (s, 2H, OH), 6.93 (d, 4H, $J = 8.4$ Hz), 2.58 (s, 6H). ^{13}C $\{^1\text{H}\}$ NMR (CDCl_3 , 400 MHz) δ : 198.2, 161.0, 131.1, 129.8, 115.5, 26.3. DEPT NMR (CDCl_3 , 400 MHz) δ : 131.2, 115.5, 26.3. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{16}\text{O}_4$: 261.1127. Found: 261.1131.

Repeating the experiment yielded a mixture of product **1** (3 mg, 1%) and a yellow solid (**2**), 4-nitrophenol (6 mg, 5%). mp 112 – 114 °C. ^1H NMR (CDCl_3 , 400 MHz) δ : 8.18 (d, 2H, $J = 9.2$ Hz), 6.91 (d, 2H, $J = 8.8$ Hz), 5.92 (s, 1H, OH), 7.07 (d, 2H, $J = 8.8$ Hz), 1.70 (s, 6H). ^{13}C $\{^1\text{H}\}$ NMR (CDCl_3 , 400 MHz) δ : 161.2, 141.8, 126.3, 115.7. DEPT NMR (CDCl_3 , 400 MHz) δ : 126.3, 115.7.

The chemical oxidation experiment was later repeated with 10% water in CH_3CN solution to give a yellow solid (**3**), 4,4'-(propane-2,2-diyl)bis(2-nitrophenol) (195 mg, 70%). mp 135 – 138 °C. IR: 3422, 1630, 1537, 1323 cm^{-1} . UV-vis: 359 nm. ^1H NMR (CDCl_3 , 400 MHz) δ : 10.53 (s, 2H, OH), 8.04 (d, 2H, $J = 2.4$ Hz), 7.33 (dd, 2H, $J = 8.8$ and 2.4 Hz), 7.07 (d, 2H, $J = 8.8$ Hz), 1.70 (s, 6H). ^{13}C $\{^1\text{H}\}$ NMR (CDCl_3 , 400 MHz) δ : 153.7, 141.7, 136.8, 133.0, 121.8, 120.2, 42.0, 30.3. DEPT NMR (CDCl_3 , 400 MHz) δ : 136.8, 121.8, 120.3, 30.3. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_6$: 319.0930. Found: 319.0929.

Products **1**, **2** and **3** obtained from chemical oxidation experiments using NOSbF_6 in CH_3CN are identified by ^1H , ^{13}C and DEPT NMR, HRMS, FTIR spectroscopy and UV-vis spectroscopy. The compound characterization data of compounds **1** – **3** are provided in the Appendix section, along with the X-ray crystal structure data of compound **3**.

TLC analysis of the reaction mixture indicated a large number of products. One product successfully isolated and purified from the reaction mixture was the hydroxylated bisdienone (**1**). Compound **1** has not previously been identified as a reaction product of BPA, possibly because the reaction conditions previously employed favored more highly oxidized products.

3.2.4 General mechanism for electrochemical oxidation of phenols

Based on the knowledge of the identity of **1**, it is reasonable to propose that a mechanism for the oxidation of BPA is through the formation of a bisdienone cation *via* two ECE steps (where E represents an electron transfer and C a chemical step) which involved the two symmetrically arranged phenolic groups.

A generic oxidative mechanism for phenols,²⁷⁻³⁰ including the well-studied examples of 2,4,6-tri-*tert*-butylphenol²⁸ and α -tocopherol,²⁹ is known to occur *via* three steps in an ECE mechanism as illustrated in Scheme 3.2. It involves two electron transfer count beginning with an initial one electron oxidation to form the phenol cation radical (E step), deprotonation of the cation radical to form neutral radical (C step) and a further one electron oxidation of the neutral radical to form the dienone cation (E step). There is however some uncertainty as to whether the reactions occur consecutively in an ECE mechanism (shown in Scheme 3.2) or whether the proton transfer step can occur concertedly with the electron transfer step such that the radical cation reacts with a phenoxyl radical to form the phenoxonium cation and the starting material in a disproportionation mechanism.³⁰

Scheme 3.2. General mechanism for electrochemical oxidation of phenols.

3.2.5 Proposal of reaction mechanism for the oxidation of bisphenol A

The overall electrochemical mechanism for the oxidation of BPA is considered to be similar to the generic oxidation pathway of phenols. It is proposed that, based on the

existing general pathway for phenols, the oxidation mechanism for BPA to form compound **1** goes through a four-electron/two-proton process ($-4e^-/-2H^+$) which is illustrated in Scheme 3.3.

Scheme 3.3. Proposed oxidative mechanism ($2 \times \text{ECE}$) for chemical oxidation of BPA with 4 NOSbF_6 in CH_3CN , consisting of four one-electron steps (E) and two-proton transfers (C). One resonance structure is displayed for each compound. The counterions for the charged ions are the chemical oxidant anion $[\text{SbF}_6^-]$ and the “ H^+ ” ions exist coordinated to the organic solvent or with trace water.

Furthermore, the four-electron transfer, which was confirmed earlier during the cyclic voltammetric monitoring of the chemical oxidation in Figure 3.3, is also in agreement with the proposed route to the formation of **1**. The exact sequence of the steps occurring during the two ECE mechanisms is complicated however because of the

presence of the two phenolic groups. Thus, the order of the electron transfer, proton transfer and hydrolysis steps may not proceed by exactly the route in Scheme 3.3. There is also the possibility of disproportionation reactions occurring where one oxidized compound undergoes homogeneous electron transfer with the starting material (which will also result in the same number of electrons transferring and the same final product).^{30,31} The adsorption effects observed during the CV experiments make it very difficult to deduce the exact sequence of proton and electron transfers *via* digital simulation modeling techniques as well.

Even though the overall oxidation involves the transfer of four electrons, only one broad anodic peak was detected. This is likely because subsequent electron transfer steps that occur after the initial electron transfer step occur at lower potentials, and this has been discussed extensively for other phenolic compounds where they are electrochemically oxidized to form products that are easier to oxidize than the starting material.^{25,28,31} The phenoxonium cation ($\mathbf{1}^+$) or dication ($\mathbf{1}^{++}$) produced during the oxidation are very unstable in the presence of nucleophiles and react immediately with moisture/water to form **1**, which is one likely reason why a chemically irreversible oxidation process was observed for BPA. Phenoxonium cations are frequently reported to be highly unstable in the presence of nucleophiles and can react immediately to undergo hydration,^{31,32} dealkylation or self-dimerization reactions with hydroxylated cyclohexadieneone being the most frequent product reported.^{27,29,31,32} Voltammetric scans performed up to 20.0 V s^{-1} did not lead to any chemical reversibility in the oxidation process as shown in Figure 3.5.

Figure 3.5. Cyclic voltammograms of 2 mM BPA with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ recorded at a 1 mm diameter GC electrode in CH_3CN at variable scan rates in an increasing order from 0.1 V s^{-1} to 20.0 V s^{-1} at $22 \pm 2^\circ \text{C}$ where the current was scaled by multiplying scan rate $^{-1/2}$. (—) First scan. (···) Second scan.

Figure 3.6 compares the cyclic voltammogram obtained for 2 mM of the chemically isolated compound **1** with the cyclic voltammogram recorded during the monitoring of the oxidation reaction as the oxidant was injected directly into the CV cell containing BPA. Both CVs have the main oxidation peak at the same potential, $\sim +1.30 \text{ V}$ vs. Fc/Fc^+ , indicating that they are likely associated with the oxidation of the same compound (**1**). The smaller peak current observed at $+1.30 \text{ V}$ for the product formed

during the electrochemical monitoring experiment was expected since the yield for compound **1** *via* chemical oxidation was substantially <100%. However, the large difference in concentration between the electrochemical experiments and chemical oxidation experiments could result in some different products being formed between the two oxidation methods. Nevertheless, electrochemical monitoring offers a fast and efficient method in investigating the electrochemical behavior of the products and their intermediate species while chemical oxidation experiments aid in the isolation of the products for identification.

Figure 3.6. Cyclic voltammograms of the products of the oxidation of 2 mM BPA with 4 molar equivalents of NOSbF_6 in CH_3CN with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ at a 1 mm GC electrode at $22 \pm 2^\circ\text{C}$ recorded at a scan rate of 0.1 V s^{-1} . (···) Electrochemically monitoring of oxidation reaction in CV cell. (—) Chemically isolated compound **1**.

The lifetime of the phenoxonium cations (Scheme 3.3, **1**⁺ or **1**⁺⁺) is partly determined by how reactive they are with nucleophiles such as water in the solvent. It is emphasized that the mechanism in Scheme 3.3 applies in the case of acetonitrile containing low amounts of water. In a strictly controlled environment or if the reaction

was performed in an entirely anhydrous solvent, the likelihood of chemically irreversible reactions such as hydrolysis is reduced which will increase the lifetime of the reactive intermediates and possibly leads to the isolation of $\mathbf{1}^+$ or $\mathbf{1}^{++}$ trapped with the nucleophilic SbF_6^- . However, chemical oxidation was performed without a strict control to stimulate electrochemical experiments. It should be noted that it is particularly challenging to fully exclude water in CV experiments unless conducted under a fully nitrogen atmosphere such as in a glovebox. In the low water content chemical oxidation conditions, compound **1** was obtained in up to 9% yield. Therefore, it was thought that increasing the percentage of water in the reaction mixture would give **1** in a higher yield. However, when the water content was increased to 10%, a nitration product (**3**) was obtained in very high yield (70%), with no evidence of compound **1**. Therefore, it would appear that the formation of **1** is highly sensitive to moisture levels that are present in the solvent. The relatively low yield of **1** that was isolated after chemical oxidation of BPA is likely to be a result of the high reactivity of all the intermediate species in Scheme 3.3 (cations, cation radicals and neutral radicals), which are able to undergo other non-hydrolysis reactions such as dealkylation and dimerization. TLC indicated that there were a number of unidentified products produced which were not easily separated from the reaction medium.

Scheme 3.4. Oxidative aromatic nitration mechanism for nitration of BPA with 2NOSbF_6 in CH_3CN modified based on an earlier report.³³

The mechanism for the formation of compound **3** in the presence of high percentages of water (Scheme 3.4) can be proposed based on an earlier report by Kim *et al.*³³ who reacted other phenolic compounds with NO^+ and suggested that the nitrosonium cation was reduced first, followed by a conversion to nitric oxide in the presence of oxygen for nitration to occur. Another nitration product (4-nitrophenol, **2**) was also obtained in low yield in low-water content solutions, likely formed by undergoing a cleavage at the methyl bridge and then reacting through the same oxidative aromatic nitration mechanism. It is also possible that the water aids the nitration reaction through hydrogen-bonding reactions with the phenolic hydrogen atom changing the reactivity of the molecule. Recently, experiments conducted to study the effect of water on the electrochemical behavior of quinones and phenols have shown that these compounds can

undergo substantial hydrogen-bonding interactions with water (even in small amounts) in organic solvents which in some cases aids in stabilization of the oxidized molecules.³⁴⁻³⁶ Figure 3.7 shows the cyclic voltammograms of different compounds **1**, **2** and **3** plotted together with the cyclic voltammogram recorded as 8 mM of oxidant was injected into the CV cell containing 2 mM of BPA.

Figure 3.7. Cyclic voltammograms of 2 mM compounds **1**, **2** and **3** isolated from chemical oxidation experiments, and a reaction mixture of 2 mM BPA + 8 mM NOSbF₆ respectively at a scan rate of 0.1 V s⁻¹ in CH₃CN with 0.2 M *n*-Bu₄NPF₆ at a 1 mm GC electrode at 22 ± 2 °C.

3.3 Conclusions

In this chapter, we have identified the long term products and intermediate species produced during the electrochemical/chemical oxidation of BPA. We also demonstrated that the phenolic environmental contaminant BPA can be oxidized in a four-electron/two-proton process to form a relatively unstable dication which reacts quickly in the presence of water in acetonitrile (in a mechanism that is similar to phenols in general).

Biological studies have shown that most compounds are not completely mineralised when ingested, but are transformed into other forms that may be more or less toxic than the parent compounds. In fact, many toxic products are produced in an oxidative metabolite pathway. The isolation of the hydroxylated bisdienone product **1** led to the proposed identity of the cationic intermediate species and this highlights the possibility of the role of the bisdienone cation as a reactive oxidative metabolite that can proceed with various reactions in biological systems (in low water content environments). More studies must be performed to investigate the toxicity of the reactive bisdienone cation species.

The one-electron chemical oxidant NO^+ has been demonstrated to be a useful alternative to electrochemical oxidation at solid electrodes for phenolic compounds that adsorb strongly on electrode surfaces for reactions conducted in organic solvents. Nevertheless, the chemical oxidation process is strongly influenced by the amount of water in the organic solvent. At low water concentrations, the oxidation process appears to occur directly *via* homogeneous electron transfer, while at higher concentrations of water, the chemical oxidant is able to take part in nitration reactions in the *ortho*-position to the phenolic group.

One complication with comparing the results of cyclic voltammetry and chemical oxidation experiments is that the CV experiments are performed at low concentrations while the chemical oxidation experiments are performed at high concentrations to enable the products of the reactions to be separated and purified. However, since we have now identified several products of the oxidation of BPA, it is possible to perform chemical oxidations at low mM levels of BPA and determine the yields of the species produced using a chromatographic technique. Future studies can also focus on quantifying how the product distribution varies as the organic solvent and the water content of the organic solvent varies.

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Chapter 4

Electrochemical Oxidation of Phenolic Benzotriazoles UV234 and UV327

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4.1 Literature Review of Chemical Studies Performed on Oxidation of Phenolic Benzotriazoles

There are a few chemical studies performed on the free radical oxidation reactions with phenolic benzotriazoles (BZTs) but the mechanistic information of the degradation steps is still not fully understood and there are different views on whether the phenolic BZTs behave in the same way as hindered phenolic antioxidants during the oxidation reactions.¹⁻⁷ Extensive studies on phenolic antioxidants have shown that they react with peroxy radicals to form intermediate phenoxy radicals, while the final products are typically peroxy cyclohexadienones and phenolic coupling products such as dimeric/polymeric phenols or quinones. The relative proportions of the final products depend on the substitution pattern of the phenol since this determines the stability of the phenoxy radicals and the steric hindrance of the approach of the peroxy radical to the phenol.²

Hodgeman *et al.*^{2,3} found that the reaction products of the phenolic BZTs (UV-P, UV-320, UV-326, UV-327) during the azo(bis)isobutyronitrile (AIBN) initiated autoxidation of cumene in chlorobenzene were similar to those obtained during the oxidation of hindered phenolic antioxidants. Cumyl-peroxy cyclohexa-2,5-dienone was identified as the major product (Scheme 4.1) and further thermal decomposition of the cyclohexadienone led to the regeneration of the starting material as well as some unidentified phenolic coupling products. It was suggested that the autoxidation mechanism of phenolic benzotriazoles involved the hydrogen abstraction from the phenol which oxidized to quinone structures, followed by addition of the peroxy radicals to the quinone. Also in another study reported by Hodgeman,⁴ a cobalt-catalyzed reaction between phenolic BZTs and the tert-butyl peroxy radical in benzene at 75 °C yielded tert-butylperoxy cyclohexa-2,5-dienone as the major product, as well as a benzotriazole

molecule which was deduced to be formed during the thermal decomposition of the cyclohexadienone. Bell *et al.*⁵ reported the formation of insoluble resinous products that was due to radical coupling reactions and not from the scission processes proposed by Hodgeman.

Gerlock *et al.*^{6,7} however, proposed that the reaction site of the phenolic BZTs did not occur at the hydroxyl group of the phenol. The photolysis of phenolic BZTs namely UV-234, UV-P and UV-328 with AIBN or cumene hydroperoxy radical in methanol, ethanol and acetonitrile solutions led to benzotriazole as the major photolytic product together with smaller fragments that had cleaved from the phenolic portion of the molecule. Results from liquid chromatography tandem mass spectroscopy supported the loss of unsaturation and alkyl substituents from the phenol group, showing clearly that the phenolic benzotriazoles did not behave like a hindered phenol antioxidant.

Scheme 4.1. Reaction mechanism proposed by Hodgeman *et al.*³ for oxidation of 2-(2'-hydroxy-5'-methyl)phenylbenzotriazole (UVP), 5-chloro-2-(3'-tert-butyl-2'-hydroxy-5'-methyl)phenylbenzotriazole (UV326) and 5-chloro-2-(3',5'-di-tert-butyl-2'-hydroxy)phenylbenzotriazole (UV327) during AIBN-initiated autoxidation of cumene.

A report published by the European Chemicals Agency (ECHA) proposed the inclusion of phenolic benzotriazoles in the candidate list of substances of very high concern for authorisation. It was concluded that the degradation *via* hydrolysis or oxidation is negligible due to the strong bonding between the benzotriazole and the aromatic ring.⁸ They also proposed that degradation through photolysis is not possible as the UV-protecting characteristics are based on a completely reversible photostabilization mechanism (described in Scheme 1.3 of Section 1.2.2.1).

While the proposed structures of the products vary across different research groups, the products showed a common intact triazole moiety, indicating that the triazole ring is relatively stable and is resistant to oxidation. There are many reports on the widespread accumulation of phenolic benzotriazoles in the environment but most analytical studies are performed as a means of quantifying the compounds and developing faster and sensitive methods for detection. As far as we are aware, there is no report on the electrochemical behavior of phenolic BZTs and their reaction pathways. In this chapter, the aim is to examine the electrochemical oxidation of UV234 and UV327 in aprotic organic solvents as well as investigate the likelihood of phenolic BZTs displaying similarity in the oxidation mechanism that is typical of most phenolic antioxidants.

2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (commercial name UV234) and 2,4-di-tert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol (commercial name UV327) were chosen mainly because their respective phenol ring consists of bulky ortho and para substituents (tert-butyl and phenyl groups) which lead to the possibility of forming stable reaction intermediates. 2,4,6-tri-tert-butylphenol is used for comparison in the discussion of the electrochemical behavior of phenolic BZTs because its molecular structure is similar to the 2,4,6-substituted structure and also because detailed mechanistic studies on the reaction pathways including the intermediates and final products have been well established.⁹

4.2 Results and Discussion

4.2.1 *Electrochemical behavior of phenolic benzotriazoles UV234 and UV327*

4.2.1.1 *Cyclic voltammetry*

The cyclic voltammograms recorded for both phenolic BZTs showed a chemically irreversible oxidation peak (E_p^{ox}) at $\sim +1.00$ V vs. Fc/Fc^+ in acetonitrile and $\sim +1.20$ V vs. Fc/Fc^+ in dichloromethane solutions as well as cathodic reduction peaks (E_p^{red}) between -2.10 V and -2.70 V vs. Fc/Fc^+ (Figure 4.1). The voltammetric responses obtained for UV234 were very similar to UV327 except for an additional small reduction peak at -2.5 V vs. Fc/Fc^+ for UV327 when recorded in CH_3CN (Figure 4.1b) which may be related to the additional chlorine atom attached to the 5-position of the benzotriazole molecule in UV327.

When the potential direction is switched after scanning past the main oxidation process at $+1.00$ V vs. Fc/Fc^+ in CH_3CN , a small reverse cathodic peak was detected at $+0.10$ V vs. Fc/Fc^+ as shown in Figure 4.1a. However, the reverse peak is so widely separated from the forward peak that it is not clear if it can be associated with the regeneration of the oxidized product back to the starting material. The absence of a clearly defined reverse peak close to the main oxidation peak indicates that the initially formed cation is short-lived and quickly reacts to form other products. The irreversible oxidation response is typical of many phenolic compounds⁹⁻¹² including bisphenol A (Chapter 3) which also display chemically irreversible oxidation peaks due to the formation of unstable oxidized compounds that quickly react to secondary oxidation products before they can be reduced back to the starting material.

As shown in the separate oxidative (Figures 4.1a and 4.1c) and reductive (Figures 4.1b and 4.1d) CVs, it is clear that the major reduction peaks are not associated with

reactions occurring after the initial oxidation processes and vice versa. Earlier reports have found only reduction processes occurring at the benzotriazole moiety. Lokesh *et al.*¹³ studied the cyclic voltammetry of benzotriazole molecule in aqueous acidic media and detected a reduction peak at -1.48 V vs. SCE . Pedersen *et al.*¹⁴ performed electrochemical reduction on substituted benzotriazoles in aprotic DMF solutions and observed two reversible peaks (E_p^{red}) for 2-phenylbenzotriazole at -1.65 V and $-2.27\text{ V vs. Ag/AgI}$. Hence, it is postulated that the oxidation and reduction peaks are independent processes occurring at different parts of the molecule, with the reduction peaks due to processes at the benzotriazole moiety. As the study was conducted to focus on the investigation of the oxidation mechanism of the phenolic BZTs, discussion on reductive CVs is omitted in the rest of the chapter. Dichloromethane is limited in its reductive potential range and this explains the detection of only one reduction peak for the reductive CVs in CH_2Cl_2 (Figure 4.1d) because any other voltammetric processes occurring more negative to $-2.5\text{ V vs. Fc/Fc}^+$ were masked by the onset of solvent reduction.

Figure 4.1. Cyclic voltammograms of 1 mM phenolic BZTs UV234 and UV327 obtained respectively at a scan rate of 0.1 V s^{-1} at a 1 mm GC electrode with $0.2 \text{ M } n\text{-Bu}_4\text{NPF}_6$ at $22 \pm 2^\circ\text{C}$ in CH_3CN and CH_2Cl_2 . (a) & (c): Oxidative cyclic voltammetry; (b) & (d): Reductive cyclic voltammetry.

4.2.1.2 Varying scan rate

Cyclic voltammograms recorded for 1 mM solutions of UV234 and UV327 at variable scan rates are displayed in Figures 4.2 and 4.3 respectively. The voltammetric peak shapes did not vary significantly over the range of scan rates tested. Voltammetric results obtained in CH_2Cl_2 solutions were very similar to the data obtained in CH_3CN , although differences in the wave shapes were expected at slower scan rates due to the higher solution resistance associated with the lower dielectric constant of CH_2Cl_2 .

Figure 4.2. Cyclic voltammograms of 1 mM UV234 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ recorded at a 1 mm GC electrode in (a) CH_3CN and (b) CH_2Cl_2 at variable scan rates in an increasing order from 0.1 V s^{-1} to 20.0 V s^{-1} at $22 \pm 2^\circ\text{C}$ where the current was scaled by multiplying scan rate $^{-1/2}$. (—) First scan. (···) Second scan.

Figure 4.3. Cyclic voltammograms of 1 mM UV327 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ recorded at a 1 mm GC electrode in (a) CH_3CN and (b) CH_2Cl_2 at variable scan rates in an increasing order from 0.1 V s^{-1} to 20.0 V s^{-1} at $22 \pm 2^\circ \text{C}$ where the current was scaled by multiplying scan rate $^{-1/2}$. (—) First scan. (···) Second scan.

Over all scan rates, the CVs performed in CH_3CN and CH_2Cl_2 showed no evidence of a reduction peak near the main oxidation peak when the scan direction was reversed. Therefore, the oxidation process appeared to remain chemically irreversible at all scan rates tested, suggesting that the intermediate oxidized form of the compounds were very short lived.

CVs were cycled twice over the potential range and as the scan rates increased to $\geq 0.5 \text{ V s}^{-1}$, new emerging peaks were detected between $0.6 \text{ V} - 0.8 \text{ V vs. Fc/Fc}^+$ along with a noticeable drop in the i_p^{ox} of the starting material during the second consecutive scan. The emerging peaks are most likely due to the formation of a new product from a follow up homogenous chemical conversion step(s) occurring after the initial

heterogeneous electron transfer. It is also possible that there is some adsorption of the oxidized species on the GC electrode which partially blocked the surface, hence leading to a corresponding decrease in the current during consecutive scanning, which has been observed previously during the electrochemical oxidation of bisphenol A (Chapter 3). The CVs obtained for UV327 in Figure 4.1a showed an overlap of the reverse current-voltage trace with the forward trace and this is typical of processes where the electrode surface has been modified such as in the case of adsorption.

4.2.1.3 *Controlled potential electrolysis and coulometry*

Controlled potential electrolysis experiments were performed on UV234 and UV327 respectively in CH₃CN by applying a potential at +1.20 V *vs.* Fc/Fc⁺. The experimental data is displayed in Figure 4.4 and Figure 4.5. During the electrolysis, the colour of both solutions changed from colourless to yellow. It was observed that both compounds showed similar changes in the CV responses although it was more difficult to electrolyze UV234 possibly due to a stronger adsorption effect on the electrode. A slightly smaller number of electrons were recorded during the electrolysis of UV234 ($n = 3.47 e^-$) compared to UV327 ($n = 3.97 e^-$).

A CV performed on the fully electrolyzed solution of UV327 (requiring the transfer of 3.97 electrons) led to the detection of an anodic peak ($E_p^{ox} = +0.55$ V *vs.* Fc/Fc⁺) and its associated reverse cathodic peak ($E_p^{red} = +0.00$ V *vs.* Fc/Fc⁺) (Figure 4.5a, red line) along with the disappearance of the peak corresponding to the starting material. A reducing potential of -0.15 V *vs.* Fc/Fc⁺ was applied to the working electrode upon the completion of the bulk oxidative electrolysis. While no evidence of the recovery of the phenol was found, the CV obtained at the completion of the reductive electrolysis showed the i_p^{ox} of the reversible anodic peak at $\sim +0.55$ V *vs.* Fc/Fc⁺ increased to a magnitude that was similar to the starting material (Figure 4.5a, blue line). Coulometry analyses made

during the reverse electrolysis of UV327 recorded a transfer of 1.74 electrons per molecule. Some other products that were reduced at negative potentials between -0.80 to -1.70 V vs. Fc/Fc^+ were also detected in the CV.

It was found that the new product formed during the bulk electrolysis at $\sim +0.55$ V vs. Fc/Fc^+ coincides with the anodic peaks detected earlier during the second scans of CV performed at scan rates $\geq 0.5 \text{ V s}^{-1}$ in Figure 4.2 and Figure 4.3. The new product was generated in greater amounts during the reverse reduction process, which indicated the complicated occurrence of multiple electron transfer and chemical reactions over the longer electrolysis timescale and this makes the proposal of a reasonable mechanism difficult simply based on the CVs obtained from electrolysis.

Figure 4.4. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV234 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ in CH_3CN at $22 \pm 2^\circ\text{C}$. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s^{-1} with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV234; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV234 at $+1.20\text{ V vs. } Fc/Fc^+$. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at $-0.15\text{ V vs. } Fc/Fc^+$.

Figure 4.5. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV327 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ in CH_3CN at $22 \pm 2^\circ\text{C}$. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s^{-1} with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV327; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV327 at $+1.20\text{ V vs. Fc/Fc}^+$. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at $-0.15\text{ V vs. Fc/Fc}^+$.

Voltammetric studies on 2,4,6-tri-*tert*-butylphenol have established a two-electron transfer count for the oxidation process.⁹ The oxidation mechanism for phenols is generally known to occur in three steps beginning with an initial $1e^-$ oxidation to form the phenol cation radical (E step), followed by a deprotonation of the cation radical to form a neutral radical (C step) and a further one electron oxidation of the neutral radical to a phenoxonium cation (E step). Phenoxonium cations are frequently reported to be highly unstable in the presence of nucleophiles and can react immediately to undergo hydration, dealkylation or self-dimerization reactions, with hydroxylated cyclohexadieneone being the most frequent product reported.^{15,16} It is possible that the oxidation mechanism for phenolic BZTs (UV234 and UV327) follows that of the phenols in an ECE mechanism to form a benzotriazolyl-substituted phenoxonium cation which then undergoes further chemical conversions to form a new species which oxidized at a lower potential than the starting material. As the potential applied for the electrolysis was relatively positive at +1.20 V *vs.* Fc/Fc⁺, the species that was produced at +0.55 V *vs.* Fc/Fc⁺ also underwent oxidation itself at the same time as it is being formed, which explains the > 2 electrons transferred during electrolysis.

A comprehensive understanding of the reaction that occurred after the formation of the phenoxonium cation is however difficult at this point mainly due to the complex reactions occurring over the long electrolysis timescale which include the formation of a new product that oxidized more easily than the starting material.

4.2.2 *Electrochemical behavior of corresponding benzotriazolyl-substituted phenolates (UV234⁻ and UV327⁻)*

4.2.2.1 *Cyclic voltammetry*

Several studies have demonstrated a simple and fast procedure of preparing phenolate anions in a 100% conversion by adding a stoichiometric amount of an organo-soluble base such as Et₄NOH to phenol solutions.^{9-11,17-22} Alkylated phenols are relatively difficult to oxidize but a large shift in the oxidation potential towards more negative values by approximately 1 – 2 V is typically observed for most phenols when they deprotonate to form their corresponding phenolates. As the phenolic BZTs are oxidized at relatively positive potentials and the use of faster scan rates did not lead to the detection of any chemical reversibility in the oxidation process as shown in Section 4.2.1, voltammetric studies were conducted in basic conditions to test for the existence of phenoxyl radicals.

Figure 4.6 shows the CVs recorded after the addition of 1 mol equiv. of an aqueous solution of *n*-Bu₄NOH to the phenolic BZTs in acetonitrile solutions where a partially chemically reversible oxidation process was immediately observed at ~ -0.2 V *vs.* Fc/Fc⁺ (red lines) at a scan rate of 0.1 V s⁻¹. There is a substantial shift in the potential of the voltammetric process, making the deprotonated phenols approximately -1.2 V easier to oxidize. The size of the anodic peak current (i_p^{ox}) is smaller than the phenols which indicated the involvement of a smaller number of electrons during the oxidation process of phenolates. As the scan was extended past the first oxidation process, it appears that the product formed from oxidizing the phenolate anions was further oxidized at more positive potentials (Figure 4.6, blue lines). A small reduction wave at ~ -1.4 V $\pm$ 0.1 V *vs.* Fc/Fc⁺ (Figure 4.6a, red lines) was detected when the scan direction was reversed after first scanning past the chemically reversible oxidation peak at -0.2 V *vs.*

Fc/Fc^+ , suggesting a follow up homogenous chemical reaction after the heterogeneous electron transfer.

Figure 4.6. Cyclic voltammograms of 1 mM UV234 and UV327 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ recorded at a 1 mm GC electrode in CH_3CN at a scan rate of 0.1 V s^{-1} at $22 \pm 2^\circ\text{C}$ over (a) small potential range; (Black lines) Phenolic BZTs; (Red lines) UV234⁻ and UV327⁻ prepared respectively by adding an equimolar amount of $n\text{-Bu}_4\text{NOH}$ to the associated phenolic BZTs; (Blue lines) Further oxidation of the phenoxyl radicals UV234[•] and UV327[•] and (b) over a wider potential range; (—) Phenolic BZTs; (···) Corresponding benzotriazolyl-substituted phenolates.

Figure 4.7. Cyclic voltammograms of 1 mM (a) UV234⁻ and (b) UV327⁻ with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm GC electrode in CH₃CN at a scan rate of 0.1 V s⁻¹ at 22 ± 2 °C scanning in the forward and reverse direction. (—) First scan. (···) Second scan.

To show that the reduction peak is indeed associated with the formation of a new product after being electrochemically oxidized, the CVs were scanned from -0.8 V vs. Fc/Fc⁺ towards the negative direction as shown in Figure 4.7; confirming that the reduction peak was only detected if the potential was first swept towards the positive potentials. A slight peak observed at -1.25 V vs. Fc/Fc⁺ in Figure 4.7b without having scanned into the anodic region most likely belongs to residual oxygen or some minor

impurities reducing at the electrode as a result of an experimental error of insufficient purging. The size of the peak is too small to be associated with the main analyte.

4.2.2.2 Varying scan rate

The effect of scan rates on the electrochemical oxidation of 1 mM solutions of UV234⁻ and UV327⁻ was tested in CH₃CN solutions (Figure 4.8) and in CH₂Cl₂ solutions (Figure 4.9).

Figure 4.8. Cyclic voltammograms of 1 mM (a) UV234⁻ and (b) UV327⁻ with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm diameter GC electrode in CH₃CN at variable scan rates in an increasing order from 0.1 V s⁻¹ to 20.0 V s⁻¹ at 22 ± 2 °C where the current was scaled by multiplying scan rate^{-1/2}. (—) First scan. (···) Second scan.

Figure 4.9. Cyclic voltammograms of 1 mM (a) UV234[−] and (b) UV327[−] with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm diameter GC electrode in CH₂Cl₂ at variable scan rates in an increasing order from 0.1 V s^{−1} to 20.0 V s^{−1} at 22 ± 2 °C where the current was scaled by multiplying scan rate^{−1/2}. (—) First scan. (···) Second scan.

The trends observed for the CVs are very similar in both CH₂Cl₂ and CH₃CN although the reduction peak at ~ −1.3 to −1.5 V vs. Fc/Fc⁺ was not detected in CH₂Cl₂ solutions due to the lower solubility in CH₂Cl₂ as a result of the difference in their dielectric constants. The oxidation process remained partially chemically reversible at all scan rates tested, which suggested that the oxidized forms of the phenolates were relatively long-lived, since even at a scan rate of 20.0 V s^{−1}, the product of the oxidation process was still detected in the form of a reduction peak at −1.4 V vs. Fc/Fc⁺ in CH₃CN (Figure 4.8). The reduction process at −1.4 V vs. Fc/Fc⁺ itself remained chemically

irreversible up to the maximum scan rate examined at 20.0 V s^{-1} . Repeated cycling resulted in the voltammetric traces closely overlaying each other unlike their corresponding phenols, indicating that adsorption was not a problem.

Figure 4.10. Overlaid cyclic voltammograms of 5 mM UV327[−] with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm diameter GC electrode in CH₃CN at variable scan rates in an increasing order from 0.1 V s^{−1} to 20.0 V s^{−1} at 22 ± 2 °C where the current was scaled by multiplying scan rate^{−1/2}.

The oxidation processes for both UV234[−] and UV327[−] displayed smaller reverse reduction peak currents (i_p^{red}) compared to their forward oxidative peak currents (i_p^{ox}) at a slow scan rate of 0.1 V s^{−1} and increasing the scan rate from 0.1 V s^{−1} to 2.0 V s^{−1} resulted in an increase in the $i_p^{\text{red}}/i_p^{\text{ox}}$ ratio to approaching unity. It was noticed however that at faster scan rates $\geq 5.0 \text{ V s}^{-1}$, the i_p^{red} of the oxidation process became smaller resulting in a drop in the $i_p^{\text{red}}/i_p^{\text{ox}}$ ratio whereas the peak at ~ -1.3 to $-1.5 \text{ V vs. Fc/Fc}^+$ increased gradually in size, more clearly observed at a concentration of 5 mM illustrated in Figure 4.10. This is consistent with a principal feature reported by Evans *et al.* in the assignment of a reversible dimerization mechanism during cyclic voltammetry experiments where the reverse cathodic peak of the oxidation process becomes smaller at faster scan rates (due to

a large k_f for the dimerization reaction), instead of becoming larger in the case for irreversible dimerization reactions.¹⁰ At slower scan rates, a reversible oxidation process of the phenolate is observed because the dimer has time to dissociate, producing phenoxyl radicals that can be reduced on the return sweep. At faster scan rates, dissociation is not fast enough which led to the detection of a large peak corresponding to the dimer species.²¹⁻²³

Figure 4.11. Plot of i_p^{ox} vs. square root of scan rate in CH₃CN ($\blacktriangle$) and CH₂Cl₂ ($\blacksquare$).

The data obtained from varying scan rates was fitted into the *Randles-Sevcik* equation,

$$i_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C^* v^{1/2} \quad (4.1)$$

where n is the number of electrons transferred, A is the electrode area in cm², C^* is the concentration in mol cm⁻³ and D is the diffusion coefficient in cm² s⁻¹. A linear relationship was obtained for the plot of oxidative peak current (i_p^{ox} in μA) against square root of scan rate (v in V s⁻¹) (Figure 4.11) with correlation values close to 1 for the CVs of both UV234⁻ and UV327⁻ which infers that the electrochemical oxidation process of the phenolic BZTs is diffusion controlled.

SWV detected distinct peaks at +1.012 V *vs.* Fc/Fc⁺ for the phenol UV234 and at −0.191 V *vs.* Fc/Fc⁺ for the phenolate UV234[−], which closely resembled that of UV327 and UV327[−] detected at +1.022 V *vs.* Fc/Fc⁺ and −0.217 V *vs.* Fc/Fc⁺ in CH₃CN. Similar peak potential values were also obtained for SWV measurements performed in CH₂Cl₂ solutions, although it was approximately 140 mV more difficult for the oxidation processes to take place (Figure 4.12). The similarity in their voltammetric behavior suggests very likely that the oxidation is taking place at a common site at the phenolic functional group.

Figure 4.12. Square-wave voltammograms of 1 mM substrates recorded at a 1 mm diameter GC electrode with 0.2 M *n*-Bu₄NPF₆ at 22 ± 2 °C in (a) CH₃CN and (b) CH₂Cl₂. (Black line) Phenolic BZTs; (Blue line) UV234[−] and UV327[−] prepared respectively by adding an equimolar amount of (1 mM) *n*-Bu₄NOH to the associated phenolic BZTs. (x) UV234; (o) UV327.

4.2.2.3 *Controlled potential electrolysis and coulometry*

Controlled potential electrolysis experiments were performed on the phenolates to accurately determine the number of electrons transferred during the oxidation process and to estimate the lifetime of the oxidized species. The applied potential was set at approximately +0.00 V *vs.* Fc/Fc⁺ for UV234⁻ (-0.05 V *vs.* Fc/Fc⁺ for UV327⁻) which was 200 mV more positive than the E_p^{ox} -value obtained by the CV measurements to ensure that a complete oxidation was achieved. The cyclic voltammograms recorded during the electrolysis of UV234⁻ and UV327⁻ in CH₃CN are shown in Figure 4.13 and Figure 4.14 respectively, along with the current *vs.* time traces obtained during electrolysis (bold lines) and the corresponding charge that has been converted into the number of electrons involved per molecule (dotted lines). Similar results recorded in CH₂Cl₂ solutions are displayed in Figures 4.15 and 4.16.

Controlled potential electrolysis of both compounds led to the detection of a large increase in the size of the reduction peak at ~ -1.45 V *vs.* Fc/Fc⁺ along with a decrease in the peak current of the starting material at -0.2 V *vs.* Fc/Fc⁺ for UV234⁻ (-0.25 V *vs.* Fc/Fc⁺ for UV327⁻) as shown in Figure 4.13a and Figure 4.14a (red lines). The exhaustive oxidation of both compounds showed that a total of one electron ($n = 1.00$ for UV234⁻ and $n = 0.98$ for UV327⁻) was transferred per molecule. Upon the completion of the oxidative electrolysis, a reducing potential of -1.65 V *vs.* Fc/Fc⁺ was applied to the working electrode to check if the oxidized species is sufficiently long-lived and if the starting material can be regenerated on the electrolysis timescale. The resultant CV obtained after the reverse electrolysis (in blue line) was identical to the CV of the phenolate (in black line) and the coulometry data confirmed a close to one electron per molecule transferred ($n = 0.81$ for UV234⁻ and $n = 0.78$ for UV327⁻) during the reverse reduction process, which is evident of a recovery of the phenolates on the electrolysis

timescale, even though there is very wide spacing between the oxidation and reduction peaks. It has been observed previously that the reverse electrolysis step does not always quantitatively regenerate the starting material even if the oxidized species is indefinitely long-lived because some oxidized product may have diffused into the other compartment of the electrolysis cell. During oxidation of the phenolates, the solution changed from a light yellow to a dark orange. During the reverse reduction, the colour of the solution changed back into light yellow. Therefore, the electrolysis experiments confirmed that the oxidized products of the phenolate anions survived in solution for at least 30 min.

The electrolysis experiments indicated that the benzotriazolyl-substituted phenolates UV234⁻ and UV327⁻ (after deprotonation of the phenolic hydrogen ion) are first oxidized by one-electron in a heterogeneous electron transfer (E step) to form a neutral benzotriazolyl-substituted phenoxyl radical. This behavior is consistent with the reports of other phenols in a basic medium. Most phenolates formed under basic conditions undergo a one-electron oxidation to form phenoxyl radical intermediates that proceed to react *via* a range of reactions mainly involving dimerization or disproportionation in aprotic solvents.^{9-11,18-22} Electrochemical studies conducted on phenoxyl radicals with bulky substituents in the 2-, 4- and 6- positions (i.e. 2,4,6-tri-*tert*-butylphenolate)⁹ have been shown to be the most stable towards irreversible bimolecular reactions, but rapid reversible reactions to form bimolecular species (dimers) are common.^{23,24}

Figure 4.13. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV234[−] with 0.2 M *n*-Bu₄NPF₆ in CH₃CN at 22 ± 2 °C. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s^{−1} with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV234[−]; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV234[−] at 0 V vs. Fc/Fc⁺. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at −1.65 V vs. Fc/Fc⁺.

Figure 4.14. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV327⁻ with 0.2 M *n*-Bu₄NPF₆ in CH₃CN at 22 ± 2 °C. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s⁻¹ with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV327⁻; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV327⁻ at -0.05 V vs. Fc/Fc⁺. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at -1.65 V vs. Fc/Fc⁺.

Figure 4.15. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV234⁻ with 0.2 M *n*-Bu₄NPF₆ in CH₂Cl₂ at 22 ± 2 °C. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s⁻¹ with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV234⁻; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV234⁻ at 0 V vs. Fc/Fc⁺. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at -1.80 V vs. Fc/Fc⁺.

Figure 4.16. Voltammetric and coulometric data obtained for the controlled potential electrolysis of 1 mM UV327⁻ with 0.2 M *n*-Bu₄NPF₆ in CH₂Cl₂ at 22 ± 2 °C. (a) Cyclic voltammograms measured at a scan rate of 0.1 V s⁻¹ with a 1 mm GC electrode: (black dotted line) prior to the bulk oxidation of UV327⁻; (red line) after the exhaustive oxidation; (blue line) after the reverse exhaustive reduction. (b) Current/coulometry versus time data obtained during the exhaustive oxidation of UV327⁻ at 0 V vs. Fc/Fc⁺. (c) Current/coulometry versus time data obtained during the reverse exhaustive reduction at -1.80 V vs. Fc/Fc⁺.

The long lifetimes of the phenoxyl radicals formed by one-electron oxidation of the phenolate anions even though the $i_p^{\text{red}}/i_p^{\text{ox}}$ ratios were $\ll 1$, can be rationalized by the radicals undergoing a reversible dimerization reaction. The detection of the new product as an increased reduction peak at $\sim -1.4 \text{ V} \pm 0.1 \text{ V}$ vs. Fc/Fc^+ along with a smaller pair of reversible waves at $\sim -0.2 \text{ V}$ vs. Fc/Fc^+ after the bulk oxidation suggested that the electrolyzed product formed is present together with the benzotriazolyl-substituted phenolate/phenoxyl radical couple (in much smaller amounts). The pair of reversible waves corresponding to the phenolate/phenoxyl radical pair was expected to be absent in the electrolyzed solution if the phenoxyl radical reacted irreversibly in a further chemical step. Therefore, it is postulated that the phenoxyl radical proceeds to react reversibly *via* a homogenous chemical step to form a product which exists in equilibrium with the phenoxyl radical rather than a simple EC mechanism (electron transfer followed by a rapid irreversible reaction).

4.2.2.4 Proposal of reaction mechanism for oxidation of phenolic benzotriazoles

The observations collated from the long lifetimes of the electrolyzed product formed after one-electron oxidation and its ability to be reversibly reduced back to the starting material *via* the same number of electrons on the electrolysis timescale raises a high possibility of a reversible dimerization reaction between the benzotriazolyl-substituted phenoxyl radicals formed *via* oxidation of the phenolates. This is supported with evidence of slow dimer dissociation mentioned in the discussion of the decreasing i_p^{red} of the oxidation process of the benzotriazolyl-substituted phenolates with increasing scan rates (Figure 4.10 of Section 4.2.2.2). EPR spectroscopy discussed in Section 4.2.3.2 also confirmed the high stability of the benzotriazolyl-substituted phenoxyl radicals and these observations are consistent with a reversible dimerization mechanism.

2,4,6-substituted phenoxyl radicals with bulky substituents at the 2- and 6- substituents have been reported to undergo decomposition mechanism such as disproportionation, reversible or irreversible dimerization.^{9,10} According to the literature, the decay route for 2,4,6-substituted phenoxyl radicals containing hydrogen atoms α to a small substituent at the 4-position is usually disproportionation to a phenol and quinone methides. In the case where an α hydrogen is absent, the phenoxyl radicals directly dimerize in a radical-radical coupling or best described as a D1M1 process to form para-quinol ethers (C–O dimers) or C–C dimers.²² Denisov *et al.* has proposed that O–O dimers are unstable and are unlikely since the high energy of stabilization of $\text{PhO}^\bullet$ due to the delocalization of the unpaired electron results in a low dissociation energy for the O–O bond.²⁴ He also suggested that phenoxyl radical dimers are weakly bonded with C–O or C–C bonds which undergo a reversible dimerization. 2,4,6-triphenylphenolate,²¹ 2,6-di-*tert*-butyl-4-methylphenolate,^{10,22} 2,6-di-*tert*-butyl-4-ethylphenolate^{10,22} and 2,6-di-*tert*-butyl-4-*n*-butylphenolate²² are examples of phenolates which after undergoing electro-oxidation form phenoxyl radicals that enter into equilibrium with the quinol ether in a reversible dimerization.

Previous studies have reported the characterisation of a C–O bonded para-quinol ether formed from reversible dimerization of 2,4,6-trialkylphenoxyl radicals^{25,26} including the σ -dimer formed from 2,6-di-*tert*-butyl-4-methylphenolate.²⁷ However, there are studies in the literature which also supported a dimerization through a π -bonded dimer for compounds that contained aromatic rings such as 9-substituted anthracene radical anions.²⁸ It has been suggested that for anion radical dimerization, the reactions giving σ -dimer are usually irreversible while the formation of π -dimers is usually fast and reversible.²⁹ We proposed that the dimer formed from the benzotriazolyl-substituted phenoxyl radicals must not be structurally complicated; the radicals should combine in a way such that the dimer can be easily dissociated into its respective phenoxyl radicals to

be able to be reduced back to the phenolate during the reverse reduction electrolysis experiments. Based on the present experimental data, it is uncertain if the dimer is π -bonded or weakly σ -bonded. Other than the commonly reported σ -dimer,²⁵⁻²⁷ it is also probable that the structure is a stable and non-covalent π stacking with multicentre long π - π interaction between singly occupied molecular orbitals of the two aromatic rings.

Scheme 4.2. Proposed oxidative mechanism (CEC_{dim}) for the electrochemical oxidation of phenolic benzotriazoles in aprotic organic solvents in basic conditions involving reversible dimerization.

The overall mechanism of phenolic BZTs UV234 and UV327 in basic conditions is proposed to be a CEC_{dim} process as illustrated in Scheme 4.2. The phenolic BZTs initially undergo a deprotonation *via* a homogeneous chemical step (C-step) to the benzotriazolyl-substituted phenolate which is oxidized *via* one-electron in a heterogeneous electron transfer (E-step) to give neutral benzotriazolyl-substituted

phenoxyl radicals that immediately undergo a reversible dimerization (C_{dim} -step) to form a dimer which exists in equilibrium with the radicals.

It is proposed that in neutral and acidic conditions, the oxidation mechanism for phenolic BZTs containing the phenolic hydrogen atom (UV234 and UV327) follows that of the phenols in an ECE mechanism to form a benzotriazolyl-substituted phenoxonium cation, A^+ (as illustrated in Scheme 4.3) *via* a two-electron/one-proton process ($-2e^-/-1H^+$). It is however difficult to ascertain if the proton coupled electron transfer occur consecutively (step-wise) or concertedly as they differ from case to case.³⁰ It is possible that the second electron transfer step occurs heterogeneously in an ECE mechanism or homogeneously in a disproportionation mechanism where the radical cation reacts with a phenoxyl radical to form the phenoxonium cation and the starting material. In basic conditions, the loss of a proton of $A \rightarrow A^-$ followed by the one-electron oxidation to $A^\bullet$ occurs in a consecutive process (Scheme 4.3).

It is noteworthy that the CV performed on the benzotriazolyl-substituted phenolate in Figure 4.6a (blue line) did lead to the detection of the oxidation peak of benzotriazolyl-substituted phenoxyl radical at a more positive potential likely *via* a one-electron process (due to their similar peak currents) to form the phenoxonium cation. This is consistent with early reports of the oxidation of phenoxyl radicals at an electrode potential apparently similar to their associated phenols.^{9,10} Therefore, it is reasonable to propose that the phenoxyl radicals produced from the one-electron electrochemical oxidation of phenolates can be further oxidized to phenoxonium cations when more positive potentials are applied. This is illustrated in Scheme 4.3 which shows the oxidation of the benzotriazolyl-substituted phenolate in two sequential one-electron steps to form the phenoxonium cation (from $A^- \rightarrow A^\bullet \rightarrow A^+$), generally typical of the oxidative mechanism for phenols in a basic medium. The formation of the phenoxonium cation product was not

expected during electrolysis of the benzotriazolyl-substituted phenolates in this study because the potential applied was very much less positive at 0 V vs. Fc/Fc^+ .

Scheme 4.3. Proposed overall mechanism for the electrochemical oxidation of phenolic benzotriazoles in aprotic organic solvents, consisting of electron transfer (E) and chemical steps (C).

4.2.3 *Spectroscopic experiments during the oxidation of phenolates*

4.2.3.1 *UV-vis spectroscopy*

Since oxidation of the phenolates produced long-lived products that could be reversibly reduced back to the starting materials, it was thought that the reactions could be usefully examined using spectroscopic analysis. The electrolyzed solutions were measured in UV-vis experiments to spectroscopically confirm that the newly formed dimer can be reduced back into the benzotriazolyl-substituted phenolate during the reverse reductive electrolysis. Figures 4.17 and 4.18 show the spectra obtained for UV234 and UV327 respectively before the start of the electrolysis (black lines), after the bulk oxidation reaction (red lines) and after the reverse reduction reaction (blue lines). In both compounds, the spectrum of the phenolates could be almost regenerated by applying a reducing potential and this is consistent with the results from the controlled potential electrolysis experiments, confirming the long lived nature of the oxidized compounds.

UV234 and UV327 displayed similar UV-vis spectra which show 2 closely spaced peaks each with their characteristic absorption maxima (λ_{max}) at 299 nm and 338 nm for UV234 and at 308 nm and 342 nm for UV327, although there are slight differences in the shapes and intensities of the bands. This is similar to most UV absorber stabilizers which typically show 2 strong absorptions of UV radiation in the 300 – 400 nm region and minimal absorbance in the visible region.⁶ Deprotonating the benzotriazolyl-substituted phenols led to a loss of their UV absorber abilities since the photostability is contributed mainly by the intramolecular hydrogen bond that exists between the phenol hydroxyl and triazole nitrogen rings and this is reflected in the UV-vis spectrum as the absorption maxima of the 2 characteristic peaks between 300 nm and 400 nm were shifted towards lower wavelengths. Two distinct peaks belonging to the UV327⁻ were observed at 250 nm and 292 nm (shifted from 308 nm and 342 nm) whereas UV234⁻ showed much

smaller bands at 251 nm and 278 nm (shifted from 299 nm and 338 nm). Other weak bands were also detected with absorption maxima at 324 nm for UV234⁻ (350 nm for UV327⁻) and 416 nm for UV234⁻ (434 nm for UV327⁻). Solutions of the phenolates were light yellow in colour while the solutions of the radicals/dimer mixture were orange.

The changes in the UV-vis spectra for UV234 benzotriazolyl-substituted phenolates throughout the electrolysis experiments were somewhat similar to UV327 benzotriazolyl-substituted phenolates due to the similarity in their structures, which indicated that both compounds were going through the same oxidative pathway. The UV-vis spectrum recorded after the bulk oxidation of UV234⁻ showed only a single strong band at 281 nm which is similar as that observed for the product of oxidation of UV327⁻ detected at 287 nm.

Figure 4.17. Background subtracted UV-vis absorption spectra of 1 mM UV234 (—) in CH₃CN containing 0.2 M *n*-Bu₄NPF₆ at 22 ± 2 °C. (···) 1 mM UV234⁻ prepared after adding 1 mM of *n*-Bu₄NOH before controlled potential electrolysis. Top graph is obtained after bulk oxidation of UV234⁻ at 0 V vs. Fc/Fc⁺ (red line) and bottom graph is obtained after bulk reverse reduction at -1.65 V vs. Fc/Fc⁺ (blue line).

Figure 4.18. Background subtracted UV-vis absorption spectra of 1 mM UV327 (—) in CH₃CN containing 0.2 M *n*-Bu₄NPF₆ at 22 ± 2 °C. (···) 1 mM UV327⁻ prepared after adding 1 mM of *n*-Bu₄NOH before controlled potential electrolysis. Top graph is obtained after bulk oxidation of UV327⁻ at -0.05 V vs. Fc/Fc⁺ (red line) and bottom graph is obtained the after bulk reverse reduction at -1.65 V vs. Fc/Fc⁺ (blue line).

4.2.3.2 EPR spectroscopy

To prove that electrolysis was indeed generating paramagnetic phenoxyl radicals, the phenolate solutions were electrochemically oxidized *via* a one-electron controlled potential bulk electrolysis and then an aliquot of the solution was transferred immediately into a silica flat cell for EPR spectroscopic analysis. The EPR spectrum was recorded every 10 min for the first hour and at a constant interval of 30 min for the next 2 h to estimate the lifetime of the radicals. A radical signal was detected for each electrolyzed solution of UV234⁻ and UV327⁻ as shown in Figure 4.19. The EPR signals of UV234[•] and UV327[•] which consisted of one single line ($\Delta H_{pp} = 4.0$ G and 4.2 G respectively) remained completely constant over a period of 3 h, confirming that the phenoxyl radicals are long-lived at room temperature.

The signal intensity was however relatively small, indicating that the radical was most likely not the major product formed from the bulk oxidation (as expected because of the reversible dimerization reaction). To determine the exact quantity of radicals in the solution, quantitative EPR experiments were conducted by comparing the signal intensity with a long-lived radical anion model produced during the one-electron reduction of 7,7,8,8-tetracyanoquinodimethane (TCNQ)^{31,32} under the same experimental conditions as the phenolate anions. The overmodulated integrated spectrum of TCNQ^{•-} was compared with the spectra of the phenoxyl radicals in order to calculate the percentage of the radicals present in the electrolyzed solutions, assuming there is no decomposition of the phenoxyl radicals and that 1 mM TCNQ^{•-} was fully converted from TCNQ. As shown in Figure 4.19, the integrated EPR absorption spectrum of both the UV234[•] and UV327[•] signals were calculated to be approximately 13% of the integrated signal intensity of TCNQ^{•-} in CH₂Cl₂, whereas both the benzotriazolyl-substituted radicals were found to be 3% of the integrated signal intensity of TCNQ^{•-} in CH₃CN. These results indicate that the

equilibrium constant for the formation of the dimer was higher (more favorable) in CH₃CN compared to CH₂Cl₂. For both solvents the diamagnetic dimer is present in larger amounts compared to the radicals in the electrolyzed mixture. This clearly shows that the equilibrium is very much in favour of the dimer and further supports the proposal of a reversible dimerization concluded earlier during the electrolysis experiments and rapid equilibrium interchanging between the radicals and dimer.

The equilibrium constant for the reversible dimerization illustrated in equation 4.2 of Scheme 4.2 can be approximated from the relative EPR signal intensities assuming that the activities are equal to concentrations. The concentration of the dimer was estimated to range from 0.87 mM – 0.97 mM depending on the solvent used and this approximated the equilibrium constant of the bimolecular dimerization reaction to be $K_{eq} \sim 10^5$ to 10^6 L mol⁻¹. EPR spectra recorded on the electrolyzed solutions obtained after the reverse one-electron reduction of the phenoxyl radicals back to the starting material did not lead to the detection of any signals.

In this section, we confirmed the existence of the benzotriazolyl-substituted phenoxyl radical with EPR spectroscopy, which was however not detected by Hodgeman *et al.*^{2,3} during their reaction of UV-327 with AIBN initiated autoxidation of cumene. We also showed that the benzotriazolyl-substituted phenoxyl radicals containing bulky substituents in the ortho and para positions remained in equilibrium with their corresponding dimers for a considerable period of time, which is also often reported in the literature for many hindered phenolic compounds.²⁴

Figure 4.19. X-band EPR spectra of a 1 mM solution of UV234⁻ (top graph) and UV327⁻ (middle graph) that had been exhaustively oxidized respectively in CH₂Cl₂ containing 0.2 M *n*-Bu₄NPF₆ *via* CPE by one-electron per molecule to form their associated radicals/dimer mixture. The bottom graph shows the EPR spectra of the electrolyzed solutions of UV234⁻ and UV327⁻ in CH₃CN and CH₂Cl₂ plotted alongside with 1 mM TCNQ that had been exhaustively reduced in CH₃CN containing 0.2 M *n*-Bu₄NPF₆ *via* CPE by one-electron per molecule to form TCNQ^{•-} in a silica flat cell at 22 ± 2 °C.

4.2.4 Chemical oxidation

Previous studies have successfully employed NOSbF_6 as a one-electron chemical oxidant to oxidize phenolic compounds and it was found that the chemical oxidation closely mimicked the results of electrochemical oxidation experiments provided that the oxidation potential was not too positive $\geq +1.0 \text{ V vs. Fc/Fc}^+$.³³ Chemical oxidation experiments were conducted on phenolic BZTs in place of electrolysis experiments to avoid the difficulties in having to remove the supporting electrolyte when trying to isolate the oxidized products under preparative scale synthetic conditions. The products of the chemical oxidation of the phenolic BZTs (UV234 and UV327) as well as their phenolates (UV234^- and UV327^-) with varying molar equivalents of NO^+ were analyzed *via* cyclic voltammetry in order to determine the equivalents of NO^+ required to achieve complete chemical oxidation and also to confirm the number of electrons transferred during the process.

The CVs obtained from the chemical oxidation experiments of both phenolic BZTs were mostly identical. In Figure 4.20a and Figure 4.21a, it was found that the size of the main oxidation peak of UV234 (as well as UV327) at $E_p^{\text{ox}} \sim +1.00 \text{ V vs. Fc/Fc}^+$ and the smaller reduction peak at $E_p^{\text{red}} \sim +0.10 \text{ V vs. Fc/Fc}^+$ remained relatively unchanged even after the addition of 2 mol equiv. of NO^+ . Unlike phenols, the associated phenolates displayed slightly different responses possibly due to their lower E_p^{ox} -values. In Figure 4.20b and Figure 4.21b, the wave belonging to the phenolate disappeared completely following the addition of 1 mol equiv of NO^+ , which indicated a transfer of 1 electron for complete oxidation. The recorded CV remained the same with the addition of a 2nd equiv. and it was confirmed that a total of 1 electron was involved in the oxidation of the benzotriazolyl-substituted phenolates which is consistent with the one-electron transfer concluded from the electrolysis experiments. Some unknown compounds, most likely a

mixture of products were also formed concomitantly to the addition of NO^+ and were detected voltammetrically at the negative potentials [~ -0.7 V and -1.9 V vs. Fc/Fc^+], which increased proportionally with increasing additions of the oxidant.

It was also observed that adding 1 mol equiv of NO^+ to the phenolate resulted in the formation of an anodic peak that oxidized at approximately the same E_p^{ox} as the phenol and with similar i_p^{ox} heights together with its corresponding reduction peak at $+0.10$ V noticed in the CVs recorded for benzotriazolyl-substituted phenols. The exact changes in the shapes of the voltammograms are difficult to explain due to the complicated homogeneous reactions that are also occurring following the electron transfer steps, but it appeared that the phenolic BZTs are regenerated on oxidation with the NO^+ . While it is possible that NOSbF_6 may not have been strong enough to oxidize the phenolic BZTs with $E_p^{\text{ox}} \sim 1.0$ V vs. Fc/Fc^+ , a more likely possibility could be due to the formation of a new nitration product that resembles closely to the starting material, explaining for the resemblance of the voltammetric peak shapes and potentials to the starting material. Unlike the successful isolation of a phenoxonium cation product for α -tocopherol^{33,34} because there were no vacant sites on the phenol ring, it is postulated that the NO^+ could be attached to either 3 vacant sites on the phenolic ring in an oxidative aromatic nitration instead. This was discussed earlier in Chapter 3 where a nitrated product was formed as one of the side products during the chemical oxidation of bisphenol A in the presence of trace amounts of water. The mechanism was proposed earlier in a study conducted by Kim *et al.*³⁵ Future studies that use NOSbF_6 for oxidation of phenols that contain vacant sites around the aromatic ring should be aware of the likely aromatic nitration reaction.

Figure 4.20. Cyclic voltammograms of 1 mM (a) UV234 and (b) UV234⁻ respectively with the addition of 1 – 2 molar equivalents of NOSbF₆ (one-electron chemical oxidant) recorded at a scan rate of 0.1 V s⁻¹ in CH₃CN at 22 ± 2 °C with 0.2 M *n*-Bu₄NPF₆ at a 1 mm diameter GC electrode.

Figure 4.21. Cyclic voltammograms of 1 mM (a) UV327 and (b) UV327⁻ respectively with the addition of 1 – 2 molar equivalents of NOSbF₆ (one-electron chemical oxidant) recorded at a scan rate of 0.1 V s⁻¹ in CH₃CN at 22 ± 2 °C with 0.2 M *n*-Bu₄NPF₆ at a 1 mm diameter GC electrode.

Synthetic scale oxidation experiments were conducted by reacting 0.1 M of UV234 and UV327 with 1 mol equiv. of base and then NOSbF₆ in a mixture of solvents CDCl₃/CD₃CN (2:1) at 293 K. A mixture of solvents was used because NO⁺ is poorly

soluble in dichloromethane and a large amount of phenolic BZTs was required which is more soluble in CH_2Cl_2 . TLC analysis of the reaction mixture indicated that there were many products formed but they were not easily separated from the reaction medium. Isolation and identification of the dimeric product from the oxidation was not successful due largely to the complex reaction mixture.

4.3 Conclusions

Earlier studies have reported findings of unidentified phenolic coupling products^{3,4} as well as insoluble resinous products⁵ from the free radical oxidation of phenolic benzotriazoles. In this study, we have shown that the benzotriazolyl-substituted phenols, UV234 and UV327 undergo a series of chemically reversible proton- and electron-transfer steps to form dimer compounds that remain in equilibrium with their corresponding phenoxyl radicals for a considerable period of time. A combination of controlled potential electrolysis and UV-vis spectroscopy experiments have shown that the radicals/dimers were stable enough to be electrochemically converted back to the phenolate forms on both the cyclic voltammetry (seconds) and electrolysis (hours) timescales. EPR spectroscopy also confirmed the existence of the UV234[•] and UV327[•] phenoxyl radicals with the relative signal intensity measurements supporting the proposal of a reversible dimerization mechanism occurring.

It is proposed that the benzotriazolyl-substituted phenols UV234 and UV327 are oxidized *via* a CEC_{dim} mechanism under basic conditions in aprotic organic solvents, showing a decay route *via* dimerization different from the stable 2,4,6-tri-tert-butylphenoxyl radicals which can survive for a long period of time in anhydrous solvents. We also reported that the main oxidation site is at the phenolic structure which is in agreement with most studies that have proposed that the benzotriazole moiety may remain unchanged under oxidative conditions.

The investigation of the voltammetry of the two benzotriazolyl-substituted phenolates UV234⁻ and UV327⁻ at glassy carbon surfaces in aprotic organic solvents indicated that the compounds are likely to undergo the same oxidation pathway as the well studied mechanism of substituted phenols which contain bulky substituents in the ortho and para positions although there is uncertainty as to whether the reactions occur consecutively or whether the proton transfer step can occur concertedly with the electron transfer step. Even though only two phenolic benzotriazoles were investigated in this study, we propose that the wide range of phenolic benzotriazoles compounds is likely to be oxidized *via* the same generic pathway for phenols in general (an ECE mechanism in neutral to acid conditions and an CEC_{dim} mechanism in basic media) considering that they contain the same 2,4,6-tri-substituted phenolic structure but differences in the reactivity of the radical or the phenoxonium cation should be expected which depends on the substituent at the ortho and para positions. Phenoxyl radicals or phenoxonium cations that contain non-bulky and less hindered substituents at their ortho and para positions most likely decompose rapidly and are difficult to study using electrochemical methods.

The high lipophilicity of the benzotriazolyl-substituted phenols combined with the findings from this study regarding the relatively high stability of the benzotriazolyl-substituted phenoxyl radicals suggest there can be serious implications on the long term toxicity and bioaccumulation effects on humans and the environment. More elaborate studies should be performed to identify the reactive metabolites in order to gain a complete understanding of their degradation pathways in both biological systems and in aquifers.

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Chapter 5

Electrochemical Oxidation of Vitamins D₃ and D₂

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5.1 Literature Review of Electrochemical Studies Performed on Oxidation of Vitamin D

While many studies can be found on the detection of vitamins D₃ and D₂, and their metabolites in food,¹⁻⁶ pharmaceuticals^{7,8} as well as in human plasma⁹ and serum samples¹⁰ with liquid chromatography^{1-7,9-11} and gas chromatography⁸ techniques, there are very few reports on the electrochemical properties of vitamin D in the literature. Among the existing electrochemical studies performed on the oxidation of vitamin D compounds, most were either reports on liquid chromatography combined with electrochemical detection used for quantitative analysis or electrode modifications to improve sensitivity and selectivity.¹²⁻¹⁵

Hart *et al.*¹⁶ studied the cyclic voltammetry of vitamins D₃ and D₂ in methanol containing acetate buffers and observed an oxidative peak (E_p^{ox}) at approximately +1.1 V vs. Ag/AgCl. Liu *et al.*¹⁷ performed CV experiments on vitamin D₂ in ethanol and detected an oxidative peak (E_p^{ox}) at approximately +1.2 V vs. Ag/AgCl on the forward scan. Mendez *et al.*¹⁸ observed an oxidative wave at +1.1 V vs. SCE (saturated calomel electrode) during differential pulse voltammetry of vitamin D₃ in methanol. Cincotto *et al.*¹⁹ observed an oxidative peak at +0.73 V vs. Ag/AgCl in 40% ethanol/60% water solution at a glassy carbon electrode during a study on the determination of vitamin D₃ in supplement tablets. It was concluded that vitamin D compounds undergo chemically irreversible voltammetric oxidation processes at glassy carbon electrode surfaces because no reduction peak was observed when the scan direction was reversed.¹⁶⁻¹⁹ A common problem reported across these electrochemical studies¹⁶⁻¹⁸ was a decrease in the oxidative peak currents (i_p^{ox}) when the potential range was cycled repeatedly due to weak adsorption of the products of the oxidation at the electrode surface.

Canevari *et al.*²⁰ developed an electrochemical sensor by modifying a glassy carbon electrode with amorphous nickel (II) hydroxide particles on a hybrid material

composed of silica and graphene oxide, SiO₂/GO/Ni(OH)₂ for the detection of vitamin D₃ and it appeared to be unaffected by adsorption effects. They studied vitamin D₃ at the modified GC electrode in 0.1 M NaOH solution at pH 13 and proposed that the oxidation occurs at the triene moiety *via* the transfer of 2 electrons resulting in the formation of diols groups (Scheme 5.1a). Although the proposed structures of the oxidized products vary, the products showed a common oxidation site at the triene (Scheme 5.1b).²¹⁻²⁷

As far as we are aware, there are few reports on the number of the electrons involved in the oxidation process and there is little mechanistic information on the oxidation pathways. In this chapter, we examine the electrochemical oxidation mechanism of vitamins D₃ and D₂ compounds in aprotic organic solvents and propose a pulsed stripping method to overcome electrode fouling caused by adsorption of the oxidized vitamin D species.

Scheme 5.1. (a) Proposed oxidation mechanism of vitamin D₂ in 0.1 M NaOH²⁰ and (b) some speculative products obtained during chemical oxidation of vitamin D₂.²⁵

5.2 Results and Discussion

5.2.1 Cyclic voltammetry

The voltammetric responses obtained for vitamin D₃ were very similar to vitamin D₂ as shown in Figure 5.1, which was also reported earlier by Hart *et al.*¹⁶ on the similarity in their behaviour. The CVs of both vitamins D₃ and D₂ using 0.2 M *n*-Bu₄NPF₆ as the supporting electrolyte in aprotic organic solvents showed one anodic oxidation peak (E_p^{ox}) at +0.8 V vs. Fc/Fc⁺, with no evidence of a reverse reduction (E_p^{red}) process (Figure 5.1). The absence of a reduction peak near the main oxidation peak suggests that the initially formed cation is reactive. Therefore, it appears that the vitamin D compounds undergo a chemically irreversible voltammetric oxidation process to form compounds that cannot be electrochemically converted back to the starting material (at least on the CV timescale of $\leq$ a few seconds).

Figure 5.1. Cyclic voltammograms of 1 mM vitamin D₃ and 1 mM vitamin D₂ recorded at a scan rate of 0.1 V s⁻¹ with 0.2 M *n*-Bu₄NPF₆ at a 1 mm diameter Pt and GC electrodes respectively in CH₃CN and CH₂Cl₂ at 22 ± 2 °C.

Square wave voltammetry led to the detection of a peak at +0.785 V vs. Fc/Fc⁺ on GC and a sharp peak at +0.801 V vs. Fc/Fc⁺ on Pt for vitamin D₂, which closely resembled that of vitamin D₃ which was detected at +0.790 V vs. Fc/Fc⁺ on GC and +0.801 V vs. Fc/Fc⁺ on Pt (Figure 5.2). SWV conducted for a solution of a mixture of 1 mM vitamin D₂ and 1 mM vitamin D₃ in CH₃CN failed to distinguish the two compounds as illustrated in Figure 5.2, but instead showed one peak at +0.816 V vs. Fc/Fc⁺ on GC and +0.826 V vs. Fc/Fc⁺ on Pt. The similarity in their voltammetric behaviour suggested that oxidation was taking place at a common site. It was proposed in previous studies that vitamins D₃ and D₂ were likely undergoing reactions at the triene moiety.²⁰⁻²⁷ Similar

peak potential values were also obtained for SWV measurements performed in CH₂Cl₂ solutions (Figure 5.2).

Figure 5.2. Square-wave voltammograms recorded at a 1 mm diameter Pt and GC electrodes respectively in CH₃CN and CH₂Cl₂ with 0.2 M *n*-Bu₄NPF₆ for three different solutions at 22 ± 2 °C. (Δ) Solution containing a mixture of 1 mM vitamin D₂ and 1 mM vitamin D₃. (○) 1 mM vitamin D₃ only. (×) 1 mM vitamin D₂ only.

5.2.2 Varying scan rate

CVs were recorded at variable scan rates ranging from 0.1 V s⁻¹ to 20.0 V s⁻¹ as shown in Figure 5.3 and Figure 5.4. The trends observed for the CVs obtained for vitamin D₃ were also very similar to that of vitamin D₂ in CH₃CN and CH₂Cl₂ at a GC electrode. The current increased with increasing scan rates as expected and the voltammetric peak shapes did not vary significantly over the range of scan rates tested.

Figure 5.3. Cyclic voltammograms of 1 mM vitamin D₃ with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm GC electrode in (a) CH₃CN and (b) CH₂Cl₂ at variable scan rates in an increasing order from 0.1 V s⁻¹ to 20.0 V s⁻¹ at 22 ± 2 °C where the current was scaled by multiplying scan rate^{-1/2}. (—) First scan. (···) Second scan.

Figure 5.4. Cyclic voltammograms of 1 mM vitamin D₂ with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm GC electrode in (a) CH₃CN and (b) CH₂Cl₂ at variable scan rates in an increasing order from 0.1 V s⁻¹ to 20.0 V s⁻¹ at 22 ± 2 °C where the current was scaled by multiplying scan rate^{-1/2}. (—) First scan. (···) Second scan.

The oxidation process for both compounds remained chemically irreversible at all the scan rates tested. No peak was observed close to the main oxidation peak on the reverse scans and this suggested that the intermediate oxidized form of the compounds was chemically short lived, likely due to a follow up homogenous chemical reaction(s) occurring after the heterogeneous electron transfer oxidation step.

CVs were cycled twice over the potential range and it was observed that the i_p^{ox} decreased noticeably during the second scans for most of the conditions tested. It is likely that the oxidized species were adsorbed on the electrode surface thereby partially blocking the surface and lowering the surface area available for oxidation and leading to a corresponding decrease in current. Previous electrochemical studies have reported similar

adsorption phenomena at glassy carbon electrodes during the oxidative scans of vitamin D.¹⁶⁻¹⁸

Plots of i_p^{ox} vs. square root of scan rate yielded straight linear graphs with correlation values of 1 and 0.999 for data collected in CH₃CN and CH₂Cl₂ respectively at the GC working electrode as shown in Figure 5.5. The linear relationship obtained infers that the electrochemical oxidation of vitamin D₃ is diffusion controlled, therefore, the adsorption effects are relatively weak and likely result in only partial blockage of the electrode surface in each scan.

Figure 5.5. Plot of i_p^{ox} vs. square root of scan rate in CH₃CN ($\blacktriangle$) and CH₂Cl₂ ($\blacksquare$).

5.2.3 Varying temperature

The CVs of vitamin D₃ were recorded at lower temperatures ranging from –40 °C to 20 °C in an attempt to increase the lifetime of the oxidized product (Figure 5.6). Decreasing peak currents were observed with lowering of temperature, which can be explained by the decrease in the diffusion coefficients, and the anodic peaks shifted towards positive potentials when the temperature was lowered. Sharper oxidation peaks

were also observed at lower temperatures since the background current was reduced considerably.

When the potential direction was switched after first scanning past the main oxidation process, temperature dependent reductive adsorption peaks were detected at approximately -0.4 V vs. Fc/Fc^+ , especially when the Pt electrode was used in CH_2Cl_2 solutions (Figure 5.6c). Adsorption peaks were more pronounced when recorded in CH_2Cl_2 solutions, which were likely due to the low solubility of the oxidized compounds. Other than the additional adsorption peaks, no other response was detected as the temperature was changed and the major oxidation process remained chemically irreversible over all measured temperatures.

Figure 5.6. Cyclic voltammograms of 1 mM vitamin D₃ with 0.2 M *n*-Bu₄NPF₆ recorded at a 1 mm diameter Pt and GC electrodes respectively in CH₃CN and CH₂Cl₂ at a scan rate of 0.1 V s⁻¹ over a range of temperatures between -40 °C to 20 °C. (—) First scan. (···) Second scan.

5.2.4 *Rotating disk electrode voltammetry*

Rotating disk electrode voltammetry was performed on vitamin D₃ to examine the voltammetric behaviour at steady state conditions. Linear sweep voltammograms recorded at scan rates of 0.02 V s⁻¹, 0.05 V s⁻¹ and 0.10 V s⁻¹ with a rotation rate of 2000 revolutions per minute (rpm) on Pt and GC electrodes in CH₃CN are shown in Figure 5.7a and 5.7c. The responses obtained were unlike the theoretically expected sigmoidal curve²⁸ when typical voltammetric scan rates at < 0.1 V s⁻¹ were used. A peak shaped response was obtained at the scan rate of 0.02 V s⁻¹ (regardless of the rotation rate), with the current increasing sharply to its maximum and then falling to the baseline instead of leveling off towards a constant limiting current. The voltammogram only became sigmoidal shaped when the scan rate was increased to 0.10 V s⁻¹ and it is likely that adsorption processes are affecting the shapes of the voltammograms. At a slower scan rate of 0.02 V s⁻¹, more time is available to attain a steady state response, but there is also more time for adsorption to occur. Therefore, even though there is a continuous supply of fresh reactants to the electrode surface, the rotating disk electrode becomes blocked by the adsorbed species. Thus, a faster scan rate of 0.10 V s⁻¹ was preferred for the hydrodynamic analysis of vitamin D₃ to reduce the effects brought about by adsorption.

Figure 5.7. Linear sweep voltammograms of 1 mM vitamin D₃ in CH₃CN with 0.2 M *n*-Bu₄NPF₆ at 22 ± 2 °C recorded with a 3 mm rotating disk Pt and GC electrodes. (a) and (c): variable scan rates of 0.02 V s⁻¹, 0.05 V s⁻¹ and 0.1 V s⁻¹ at 2000 rpm; (b) and (d): variable rotation rates of 500, 1000, 1500, 2000 and 2500 rpm at 0.1 V s⁻¹.

Linear sweep voltammograms of solutions of vitamin D₃ were recorded with a 3 mm diameter rotating disk Pt and GC electrode at a scan rate of 0.1 V s⁻¹ using a variation of five rotation rates (Figure 5.7b and 5.7d). The general reversible wave equation²⁹ is often used to test for reversibility under steady state conditions of a hydrodynamic voltammogram and can be written in the form

$$E = E_{1/2} - 0.0592/n \log \{ [i_d(\tau) - i(\tau)] / i(\tau) \} \quad (5.1)$$

where E is the potential and $E_{1/2}$ is the half-wave potential in volts, n is the number of electrons transferred, $i_d(\tau)$ is the limiting current and $i(\tau)$ is the current for a fixed sampling time τ . An electrochemically reversible system yields a linear plot with a slope of $-59.2/n$ mV at 25 °C when E is plotted against $\log [(i_d - i)/i]$. Experimental values recorded for the five rotation speeds at a scan rate of 0.1 V s⁻¹ at Pt RDE shown in Figure

5.7b were arranged in logarithm form, $\log [(i_d - i)/i]$ and plotted with E using the linear least squares method to give straight line graphs.

Figure 5.8. Wave slope analysis of steady state voltammograms. (a) Linear sweep voltammogram of 1 mM vitamin D₃ obtained at a scan rate of 0.1 V s^{-1} in CH_3CN with $0.2 \text{ M } n\text{-Bu}_4\text{NPF}_6$ at 22 ± 2 °C with a 3 mm rotating disk Pt electrode recorded at 500 rpm. (b) Plot of E/V vs. $\log[(i_d - i)/i]$ using experimental data recorded for (a).

An example of the data analysis for the response obtained at scan rate of 0.1 V s^{-1} with a 3 mm Pt electrode rotated at 500 rpm in CH_3CN is displayed in Figure 5.8. The slope obtained was approximately -60.4 mV , translating to a value close to 1 for n . The calculated values of n ranged from 0.64 to 0.98 for the data collected at Pt and GC electrodes in CH_3CN (Table 5.1). It should be noted that there could be some calculation errors made during the measurements of the $\log [(i_d - i)/i]$ values as the hydrodynamic response given was deviating from the ideal sigmoidal behaviour. Instead of reading

directly off an ideal sigmoidal voltammogram for a maximum limiting current, the i_d was determined at the intersection formed by two lines extrapolated from the i vs. E plot. The potential at which the limiting current is measured is at +0.98 V vs. Fc/Fc⁺ (at $i_d = 55 \mu\text{A}$) in this example in Figure 5.8a). Determination of the exact value of the number of electrons transferred during the oxidation process was made difficult by the presence of adsorption on electrode surfaces. Nonetheless, the data seemed to agree that this is a one-electron transfer process since n values were calculated to be close to 1 in most instances.

Table 5.1. n values obtained from wave slope analysis.

Electrode	3 mm GC RDE		3 mm Pt RDE	
<i>Rotation rate (rpm)</i>	<i>Gradient</i>	<i>n</i>	<i>Gradient</i>	<i>n</i>
500	-0.066	0.90	-0.060	0.98
1000	-0.075	0.79	-0.065	0.92
1500	-0.078	0.76	-0.070	0.85
2000	-0.080	0.74	-0.071	0.84
2500	-0.092	0.64	-0.081	0.73

Using the Levich equation (discussed in Section 2.2.4), a plot of i_l against $\omega^{1/2}$ yielded a linear graph with gradient of $7.142 \times 10^{-6} \text{ A (rad s}^{-1})^{-1/2}$ that can be used to solve for the diffusion coefficient of vitamin D₃ ($D_0 = 1.817 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) during the oxidation process in CH₃CN at a 3 mm rotating disk Pt electrode as shown in Figure 5.9. The diffusion coefficient obtained was typical of molecules with a molecular mass around 400 g mol⁻¹. Unlike the test for reversibility, the calculation carried out for the diffusion coefficients is independent of the slope as it only takes into account the limiting current. Hence, the diffusion coefficients of the analyte can still be determined despite the deviation (provided the n -value is correct).

Figure 5.9. Plot of i_l vs. $\omega^{1/2}$ for data collected in CH₃CN at a scan rate of 0.1 V s⁻¹ with a 3 mm rotating disk Pt electrode.

5.2.5 Controlled potential electrolysis and coulometry

CH₂Cl₂ was used as the solvent for controlled potential electrolysis experiments because a large amount of the analyte was required and vitamin D compounds were more soluble in CH₂Cl₂ compared to CH₃CN. Moreover, relatively sharper oxidation peaks were observed for CVs recorded in CH₂Cl₂ solutions. To ensure complete oxidation of the vitamin D compounds, the potential was applied at +1.0 V vs. Fc/Fc⁺ which was approximately 200 mV more positive than the E_p^{ox} -value obtained by the CV measurements.

A first attempt to electrolyze vitamin D using the conventional (1-step) electrolysis procedure was not successful. The current was observed to decrease sharply to extremely low levels and the amount of electrical charge passed was found to increase at a very slow rate after ~ 700 seconds such that electrolysis appeared to be coming to a stop (Figure 5.10a). The low charge passed was due to fouling problems at the Pt mesh working electrode. A CV performed of the electrolysis solution at a 1 mm Pt electrode led to the detection of a new anodic peak at a lower potential than the starting material's oxidation peak, indicating that electrolysis was producing a product that is easier to

oxidize than the starting material. As the electrolysis progressed, the i_p^{ox} of the new emerging peak at $\sim +0.6$ V vs. Fc/Fc^+ increased whereas the peak associated with oxidation of the starting material diminished (Figure 5.10b). Analysis of the coulometric data however indicated a transfer of 0.62 electrons over 4350 seconds. CPE experiments were conducted at lower temperatures but were not successful. In fact, the electrolysis current diminished even faster at 0 °C, suggesting that adsorption effects were more pronounced at temperatures lower than room temperature, which was also experienced when performing CV in low temperatures (Figure 5.6).

Figure 5.10. Experimental data obtained for the controlled potential electrolysis (without pulsed stripping) of 5 mM vitamin D₃ with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ in CH_2Cl_2 at 22 ± 2 °C. Cyclic voltammograms measured at a scan rate of 0.1 V s^{-1} with a 1 mm Pt electrode before (---) and after (—) (a) a period of 750 s and (b) an additional period of 3600 s continuing from part (a) and their corresponding current vs. time graphs.

An additional cathodic pulsed stripping step was adopted to overcome the problem of electrode fouling from the adsorption of oxidized products on the electrode surface as

the electrolysis proceeded. The electrolysis potential was switched to $-1.0\text{ V vs. Fc/Fc}^+$ for vitamin D₃ ($-0.9\text{ V vs. Fc/Fc}^+$ for vitamin D₂) for $\sim 30\text{ s}$ when the electrolysis current was observed to drop below 1 mA at the large Pt mesh working electrode (which was used as a gauge that the electrode had fouled; minimal current was passing) and it was observed that the adsorbed species were stripped off the electrode during the reduction process. This pulse stripping step was incorporated into the bulk electrolysis of vitamin D which involved alternating several cycles of oxidative electrolysis and reductive stripping and this proved to work effectively. The current flowing through the cell was able to sustain longer periods before it dropped to values below 1 mA .

Cyclic voltammograms recorded throughout the electrolysis of vitamin D₃ and vitamin D₂ using the pulsed stripping method are shown in Figure 5.11 and Figure 5.12 respectively. Similar voltammetric responses were observed for the electrolysis of both vitamins D₃ and D₂. Controlled potential electrolysis of both compounds led to the detection of a new oxidative peak at $\sim +0.6\text{ V vs. Fc/Fc}^+$ which initially increased in intensity as electrolysis proceeded, along with a decrease in the i_p^{ox} of the starting material at approximately $+0.8\text{ V vs. Fc/Fc}^+$. Eventually the species responsible for the peak at $+0.6\text{ V vs. Fc/Fc}^+$ also underwent oxidation itself and its associated oxidative peak decreased in magnitude. The total number of electrons transferred during the bulk electrolysis experiment was calculated to be 2.16 for vitamin D₃ and 2.08 for vitamin D₂ after approximately 3 h.

Figure 5.11. Cyclic voltammograms recorded at a scan rate of 0.1 V s^{-1} with a 1 mm Pt electrode during the controlled potential electrolysis of 5 mM vitamin D₃ in CH_2Cl_2 with 0.2 M $n\text{-Bu}_4\text{NPF}_6$. The controlled potential electrolysis experiment was performed by incorporating a pulsed stripping step at $-1.0 \text{ V vs. Fc/Fc}^+$ for 30 s in between oxidative electrolysis runs in attempts to overcome electrode fouling. n is the apparent number of electrons transferred during electrolysis; (dotted black lines) before bulk oxidation, (red lines) after bulk oxidation conducted for over 3 h.

Figure 5.12. Cyclic voltammograms recorded at a scan rate of 0.1 V s^{-1} with a 1 mm Pt electrode during the controlled potential electrolysis of 5 mM vitamin D₂ in CH₂Cl₂ with 0.2 M *n*-Bu₄NPF₆. The controlled potential electrolysis experiment was performed by incorporating a pulsed stripping step at $-0.9 \text{ V vs. Fc/Fc}^+$ for 30 s in between oxidative electrolysis runs in attempts to overcome electrode fouling. n is the apparent number of electrons transferred during electrolysis; (dotted black lines) before bulk oxidation, (red lines) after bulk oxidation conducted for over 3 h.

5.2.6 Proposal of reaction mechanism for the oxidation of vitamin D

The CV experiments have shown that both vitamin D compounds undergoing a one-electron oxidation on the short cyclic voltammetric timescale ($t \leq$ seconds). However, on the longer electrolysis timescale ($t \geq$ minutes), it provided ample time for multiple electron transfer and chemical reactions to take place which allows the detection of peaks belonging to intermediate species and/or the final electrolysed product that were previously not observed. Therefore, it is proposed that both compounds undergo electrochemical oxidation on the long voltammetric timescale *via* multiple electron and chemical steps.

Scheme 5.2. Proposed oxidative mechanism (ECCE) for the electrochemical oxidation of vitamin D in aprotic organic solvents, consisting of electron transfer (E) and chemical steps (C).

Vitamin D is first oxidized by one-electron in a heterogeneous electron transfer (E) to give the cation radical which reacts quickly in a homogeneous chemical step (C-step) to form a new compound. The new compound then undergoes an additional chemical conversion (C-step) to form a new species which is oxidized at a lower potential than the starting material (and is detectable during the electrolysis experiments). The overall mechanism can be depicted as an ECCE process, but with further chemical steps also possible after the second electron transfer (Scheme 5.2).

It is thought that the first chemical step occurs relatively quickly, since no chemical reversibility is detected in the cyclic voltammograms even at moderately fast scan rates. However, the second chemical step likely occurs more slowly, otherwise the

product that is formed (that oxidized at 0.2 V more negative than the starting material) would also undergo immediate oxidation during CV and electrolysis experiments. Instead, the product formed in the second chemical step forms sufficiently slowly so that it is not immediately oxidized during electrolysis experiments and is able to leave the electrode surface and move into the bulk solution where it is detected by CV in Figures 5.11 and 5.12.

5.2.7 Chemical oxidation

The chemical oxidation of vitamins D₃ and D₂ with varying molar equivalents of NO⁺ was first analyzed *via* cyclic voltammetry, in order to determine the equivalents of NO⁺ required to achieve complete oxidation, and confirm the number of electrons transferred during the process. The CVs obtained from the chemical oxidation of both vitamins D₃ and D₂ were almost identical. It was found that the size of the main oxidation peak of vitamin D at $E_p^{ox} = +0.8$ V vs. Fc/Fc⁺ was approximately reduced by half after the addition of the 1st mol equiv. of NO⁺ (Figure 5.13). The wave belonging to the starting material disappeared completely following the addition of 2 mol equiv of NO⁺, and this confirmed the transfer of two electrons for exhaustive oxidation. The recorded cyclic voltammogram of the product remained the same with the addition of a 3rd equivalent and it was hence concluded that a total of 2 electrons was involved in the oxidation of the vitamins. Some unknown compounds, likely a mixture of products were also formed concomitantly to the addition of NO⁺ and were detected voltammetrically at the negative potentials [~ -0.3 V and -1 V vs. Fc/Fc⁺], which increased proportionally with increasing additions of the oxidant (Figure 5.13). The exact changes in the shapes of the voltammograms are difficult to explain due to the complicated homogeneous reactions that are also occurring following the electron transfer steps.

The two equivalents of NO^+ needed to oxidize vitamins D₃ and D₂ supports the findings concluded earlier from CPE experiments on the number of electrons transferred during the oxidation process of vitamin D as well as the proposal of an ECCE mechanism. The similarity in the electrochemical responses strongly suggested that the two vitamin D compounds are sharing a common oxidation site.

Figure 5.13. Cyclic voltammograms of 2 mM (a) vitamin D₃ and (b) vitamin D₂ respectively with the addition of 1 – 3 molar equivalents of NOSbF_6 (one-electron chemical oxidant) recorded at a scan rate of 0.1 V s^{-1} in CH_3CN at $22 \pm 2^\circ \text{C}$ with $0.2 \text{ M } n\text{-Bu}_4\text{NPF}_6$ at a 1 mm diameter GC electrode.

Synthetic scale oxidation experiments were conducted by reacting 0.156 M of vitamin D₃ with 2 mol equiv. of NOSbF₆ in a mixture of solvents CH₂Cl₂/CH₃CN (4:1). A mixture of solvents was used because NO⁺ is poorly soluble in CH₂Cl₂ and a large amount of vitamin D was required which is more soluble in CH₂Cl₂. Thin layer chromatography analysis of the reaction mixture indicated that there were a few products formed but they were not easily separated from the reaction medium. The products were not easily isolated and purified for analysis, thus only crude ¹H NMR spectra were obtained for analysis. A comparison with the NMR spectrum of the starting material showed that the alkene peaks at approximately 5 and 6 ppm were absent from the spectra of the oxidized products, indicating that the triene was the likely site of oxidation as illustrated in Figure 5.14. The NMR spectra obtained however did not match the diol product found by Toh *et al.*²¹ It is difficult to propose an identity for the oxidized products based on the (overlapping alkyl region of the) NMR spectra. The product mixture was very sensitive to light and moisture, making the collection of other characterisation not possible.

¹H spectrum of the starting material vitamin D₃ is shown in Figure 5.14. ¹H NMR (CDCl₃, 400 MHz) δ: 6.24 (d, 1H, 11.2 Hz), 6.03 (d, 1H, J = 11.6 Hz), 5.05 (s, 1H), 4.82 (d, 1H, J = 2.4 Hz), 3.95 (s, broad, 1H, OH), 2.82 – 0.54 (alkyl H, 39H).

NOSbF₆ (2 equiv) was dissolved in 1 mL of CH₃CN and then added to a solution of vitamin D₃ (300 mg, 0.156 mol) in 4 mL of CH₂Cl₂ at 22 ± 2 °C. The solution became dark brown and was stirred for another 30 min while checking with TLC for complete reaction. The solvent was then removed under vacuum and the residue was separated by column chromatography and preparative thin layer chromatography. The chemical oxidation experiments were repeated thrice. **(1)** Crude ¹H NMR (CDCl₃, 400 MHz) δ: 8.06 (t, 1H, 1.6 Hz), 7.97 (d, 1H, J = 7.6 Hz), 7.57 (d, 1H, J = 7.2 Hz), 7.41 (t, 1H, J = 8 Hz), **(2)** Crude ¹H NMR (CDCl₃, 400 MHz) δ: 8.06 (s, 1H), 7.96 (d, 1H, J = 8 Hz), 7.55

(t, 1H, $J = 10.4$ and 7.2 Hz), 7.40 (t, 1H, $J = 8$ and 7.6 Hz), (**3**) Crude ^1H NMR (CDCl_3 , 400 MHz) δ : 7.71 (d, 1H, 9.2 Hz), 7.53 (d, 1H, 8.4 Hz), 7.00 (s, 1H). Crude ^1H spectra obtained from chemical oxidation experiments using NOSbF_6 in $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$ are provided in the Figures 5.15 – 5.17.

Figure 5.14. Proposed oxidation site of vitamin D₃ and D₂.

Figure 5.15. ^1H NMR spectrum of vitamin D₃ (CDCl_3 , 400 MHz).

¹H NMR, BBFO1, 400 MHz

Figure 5.16. ¹H NMR spectrum (CDCl₃, 400 MHz) of vitamin D₃ oxidation products from chemical oxidation reaction (1).

¹H NMR, BBFO1, 400 MHz

Figure 5.17. ¹H NMR spectrum (CDCl₃, 400 MHz) of vitamin D₃ oxidation products from chemical oxidation reaction (2).

Figure 5.18. ¹H NMR spectrum (CDCl₃, 400 MHz) of vitamin D₃ oxidation products from chemical oxidation reaction (3).

5.3 Conclusions

The investigation of the voltammetry of vitamins D₃ and D₂ at glassy carbon and platinum electrode surfaces in aprotic organic solvents indicated that the compounds displayed similar electrochemical response which is related to the similarity in their structures. Voltammetric scans performed up to scan rates of 20 V s⁻¹ as well as at lower temperatures (down to -40 °C) did not lead to the detection of any chemical reversibility of the oxidation process. The oxidation process was diffusion controlled, indicating that a fast electron transfer is occurring across the interface, albeit with evidence of weak adsorption on the short voltammetric timescales.

CPE experiments performed by incorporating several cycles of oxidative electrolysis and pulsed cathodic stripping steps were useful in overcoming the problem of electrode fouling. It was proposed that the electrochemical oxidation of vitamin D

involved one electron per molecule on the short cyclic voltammetry timescale ($t \leq$ seconds) and two electrons per molecule during the longer timescale ($t \geq$ minutes) controlled potential electrolysis experiments, suggesting that vitamin D is oxidized *via* an ECCE mechanism – an initial one electron oxidation to form an electroinactive product which reacts *via* coupled homogeneous chemical reactions to form a product that undergoes heterogeneous oxidation at a lower potential than the starting material. The transfer of 2 electrons per molecule over longer timescales was also supported by chemical oxidation experiments with NO^+ . NMR spectroscopic analysis of the chemical oxidation products indicated that it was very likely that the oxidation reaction occurred at the triene moiety.

Although the incorporation of the pulsed stripping step has proved to be effective in reducing the adsorption effects for achieving exhaustive electrolysis, a comprehensive understanding of the oxidation mechanism is still difficult at this point mainly due to the complications arising from adsorption on the electrode surfaces as well as the many complex reactions occurring over the longer electrolysis timescale including the formation of a new product that is oxidized more easily than the starting material. However, it is recommended to incorporate pulsed stripping to overcome the problem of electrode fouling during the electrochemical detection of vitamin D compounds when conducting chromatographic analysis in test samples.

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Chapter 6

Electrochemical Oxidation of Bisphenol A, Phenolic Benzotriazole UV327 and Vitamin D₃ at MWCNT-coated Electrodes & Assessment of Surface Coverage after Nanomaterials are Drop Cast onto Electrodes

6.1 Introduction

- 6.1.1 *Electrode fouling and review of electrochemical studies performed at electrode surfaces modified with nanomaterials*
- 6.1.2 *Electrochemical oxidation of artificial and natural hydroxyl containing compounds at MWCNT-coated electrodes*
- 6.1.3 *Assessment of surface coverage after nanomaterials are drop cast onto electrodes*

6.2 Results and Discussion

- 6.2.1 *Voltammetric behavior of Cu electrodes modified with porous layers of MWCNTs*
- 6.2.2 *SEM-EDX analyses of Cu electrodes modified with porous layers of MWCNTs*
- 6.2.3 *Voltammetric behavior of GC electrodes modified with porous layers of MWCNTs*
- 6.2.4 *Voltammetric behavior of GC electrodes modified with nano-sized alumina powder*

6.3 Conclusions

References

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6.1 Introduction

6.1.1 *Electrode fouling and review of electrochemical studies performed at electrode surfaces modified with nanomaterials*

In chapters 3 – 5, the voltammetric behavior of bisphenol A, phenolic benzotriazoles and vitamin D compounds were examined respectively with a range of electrochemical oxidation methods. During the electrochemical oxidation of the three hydroxyl containing compounds, we encountered fouling problems at the bare electrode surfaces especially with BPA and vitamin D. The adsorption processes resulted in diminishing oxidative peak currents (i_p^{ox}) with repetitive scanning and partially blocked the electrode surfaces for further analysis. This is undesirable for electrochemical studies as it impedes the determination of the number of electrons transferred and the investigation of the oxidation mechanism, especially during long timescale controlled potential electrolysis experiment which does not allow continuous polishing to renew the electrode surface.

In the literature discussed in the respective sections, some studies have reportedly achieved good selectivity and sensitivity in their electrochemical responses by modifying electrode surfaces with nanomaterials. Many nanoscale materials based electrochemical sensors have been developed in recent years including the single-walled carbon nanotubes/poly{3-butyl-1-[3-(N-pyrrolyl)propyl] imidazolium ionic liquid} composite film modified electrode,¹ core-shell multiwalled carbon nanotubes/graphene oxide nanoribbons,² polyglutamic acid/amino-functionalised carbon nanotubes nanocomposite,³ ordered mesoporous carbon modified nano-carbon ionic liquid paste electrode,⁴ diazonium-functionalized boron-doped diamond electrode modified with a multi-walled carbon nanotube-tyrosinase hybrid film,⁵ molybdenum disulfide nanoflower-chitosan-Au nanoparticles composites⁶ for detecting BPA and a modified glassy carbon electrode with

amorphous nickel (II) hydroxide particles on a hybrid material composed of silica and graphene oxide, $\text{SiO}_2/\text{GO}/\text{Ni}(\text{OH})_2$ for detecting vitamin D_3 .⁷

Nanoscale carbonaceous materials such as carbon nanotubes (CNTs) and graphene sheets have been widely studied for electrode modifications and research into developing new porous nanostructured electrodes is expanding rapidly. Cited attractive properties include high aspect ratios, excellent electrical conductivity, chemical stability in acidic and basic solutions, and mechanical strength.⁸⁻¹⁹ These nanomaterials are typically used in the form of films cast from a suspension onto bare electrode surfaces which result in a high surface area. There are many methods adopted for modifying electrodes but drop casting is a common approach as evidenced from the wide usage in literature reports due to the low cost and ease of preparation.^{1-11,17,20,21} The technique was first described by Liu *et al.*²² where CNTs were dispersed in a suitable solvent and an aliquot was then dropped onto the electrode surface to evaporate. Due to the large aspect ratio, CNTs are subject to large van der Waals attractive forces and have a high tendency to aggregate and form large bundles. It is difficult to achieve a stable and homogenous suspension without using covalent chemistry or harsh treatments which can lower the electrical conductivity.^{9,10} Dispersing is performed with a non polar organic solvent and dimethylformamide (DMF) or *N*-methyl-2-pyrrolidone (NMP) are widely regarded as the best suitable solvents for nanotube dispersion.²³

CNTs are routinely used for modifying electrodes because of frequent reports on their providing improved electrochemical properties. These studies have observed an increase in electron transfer rates (measured as smaller forward and reverse peak-to-peak separations during cyclic voltammetry measurements, or enabling easier oxidation or reduction for irreversible processes), decreased overpotentials and an increase in the voltammetric peak currents which allows lower detection limits and enhanced sensitivity in electroanalytical detection with little electrode fouling.¹⁷⁻¹⁹

6.1.2 *Electrochemical oxidation of artificial and natural hydroxyl containing compounds at MWCNT-coated electrodes*

The electrochemical oxidation of bisphenol A, phenolic benzotriazole UV327 and vitamin D₃ were investigated with a GC electrode modified with carbon nanotubes to attempt to overcome the effects of electrode fouling and increase the peak currents. Prior to drop casting, the GC electrode was cleaned by polishing with an alumina oxide slurry with a grain size of 0.3 μm on a polishing cloth, sonicated in water, then ethanol and then in 0.5 M HNO_3 for 5 min each. Multiwalled carbon nanotubes (MWCNTs) were applied to the electrode by repeatedly coating the electrode with 0.5 μL droplets of the MWCNTs suspended in DMF. Each droplet was allowed to dry before the next application.

A 1 mm diameter bare GC electrode was modified with 1 – 8 droplets of MWCNTs drop casting solution and studied in CH_3CN containing 1 mM ferrocene (Figure 6.1). Fc was used as a redox standard in organic solvents as it displays ideal electrochemical reversible behavior. An enhancement in the voltammetric peak currents was observed for the CVs recorded at the modified MWCNT-coated GC electrode. The CVs recorded at the GC electrode modified with 8 layers of MWCNTs in Figure 6.1 observe up to an eight-fold increase in the i_p^{ox} of ferrocene as compared to the bare GC electrode, most likely due to an increase in the surface area of the electrode. There is however no improvement in the peak-to-peak separation. The results seemed to suggest that larger voltammetric peak currents can be achieved simply with two drop coated layers although there was no notable trend between the number of MWCNT layers and the size of the peak currents.

Figure 6.1. Cyclic voltammograms of 1 mM ferrocene recorded at a scan rate of 0.1 V s^{-1} at a 1 mm GC electrode in CH_3CN solution with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ at $22 \pm 2^\circ\text{C}$. The black line represents the bare GC electrode and the colored lines represent the GC electrode coated with 1 droplet (blue), 2 droplets (green), 4 droplets (brown), 6 droplets (red) and 8 droplets (grey) of the MWCNT casting solution.

The electrochemical behavior of bisphenol A, phenolic benzotriazole UV327 and vitamin D_3 were investigated at the 1 mm GC electrode modified with 1, 2 and 4 layers of MWCNTs and the CVs responses are illustrated in Figure 6.2. Although the voltammetric peak currents of the oxidation processes increased as expected at the modified electrodes, the responses differed when tested with different compounds.

During the investigation at the modified GC electrode, the oxidation peak detected at $+1.0 \text{ V vs. Fc/Fc}^+$ for UV327 as well as its reverse reduction peak detected at $+0.1 \text{ V vs. Fc/Fc}^+$ when the potential was switched after scanning past the main oxidation peak became more distinct as compared to studying at the bare electrode (Figure 6.2b). A small reduction peak appeared at $\sim +0.05 \text{ V vs. Fc/Fc}^+$ for BPA on the reverse scan which was not detected previously at the bare electrode in CH_3CN (Figure 6.2a).

However, in contrary to other studies showing improved sensitivity with no reported fouling problems with the electrode, i_p^{ox} for all three compounds remained smaller during the second consecutive scans compared to the first scans, suggesting that the oxidized species were still adsorbing to the modified MWCNT-coated GC electrode. There was a 20 times increase in the i_p^{ox} for BPA (Figure 6.2a), 14 times increase for vitamin D₃ (Figure 6.2c) and 8 times increase for UV327 (Figure 6.2b) from the bare GC electrode (black lines) to the GC electrode modified with 8 layers of MWCNTs (coloured lines). It is possible that the increase in i_p^{ox} is related to the extent of the adsorption of the oxidized species on the electrode.

As discussed in Chapters 3 – 5, BPA could not be electrolysed due to the fouled working electrode from the adsorption effect while electrolysis worked for vitamin D₃ (requiring several cycles of pulsed stripping) and UV327 (no pulsed stripping required). It appeared that the analyte molecules may have diffused through the MWCNTs to adsorb on the bare electrode surface and/or trapped in between the porous nanotube layers, thus giving rise to larger i_p^{ox} values especially for analytes that have been reported to adsorb strongly. There is also uncertainty to the number of MWCNTs layers required for coating the electrode to ensure that the oxidation process is occurring only at the drop cast nanomaterials. It appeared that the drop casting was creating randomly distributed films as there was no specific trend between the number of MWCNT layers and the size of the peak currents.

Figure 6.2. Cyclic voltammograms of (a) 2 mM BPA, (b) 1 mM UV327 and (c) 2 mM VD_3 recorded at a scan rate of 0.1 V s^{-1} at a 1 mm GC electrode in CH_3CN solution with 0.2 M $n\text{-Bu}_4\text{NPF}_6$ at $22 \pm 2^\circ\text{C}$. The black line represents the bare GC electrode and the colored lines represent the GC electrode coated with 1, 2 and 4 droplets of the MWCNT casting solution. (—) First scan. (···) Second scan.

6.1.3 *Assessment of surface coverage after nanomaterials are drop cast onto electrodes*

There are different views regarding the origins of the improved electrochemical responses of CNT-coated electrodes. Some groups have focused their research on utilizing the favorable properties including the inherent enhancement in the electron transfer kinetics in applied applications, but there are also studies suggesting alternative factors are responsible for the apparent excellent performance.^{8,9,24,26,28,29} The controversy regarding their excellent electrochemical properties has been raised in review papers^{8,25-28} and there are several theories/models proposed to explain for the potential misinterpretation of electrocatalysis at CNT-modified electrode systems.^{23,30-47} A simple alternative explanation for apparent electrocatalysis is often given by the increase in the electrochemical active surface area of the porous working electrode from the high surface area nanotube film resulting in larger current values.

Besides the increased surface area, there has also been discussion on the effects of the electrode morphology. Some theoretical simulations and experimental work by Compton *et al.* have reported characteristics of a faster electron transfer (i.e. peak current and peak-to-peak separation) after surface modification of the electrode with a porous layer.^{30,31,43,44} A recent study conducted by Punckt *et al.*³³ also mentioned that minute amounts of electrode porosity can cause significantly faster apparent electrode kinetics as determined by cyclic voltammetry, both in the reversible and irreversible regime. The reported electrocatalytic properties are also attributed to residual metallic impurities³⁴ and nanographitic impurities added during the manufacturing process of carbon nanotubes,^{35,36} electroactive functional groups,³⁷ defects in the edge-plane sites and tube ends³⁸⁻⁴¹ for carbon nanotubes or at the edges of the sheets for graphene-based electrodes⁴² which act as active sites of electrochemical reaction.

Recently, it was suggested that a thin film diffusion-related effect must be taken into account for the correct interpretation of the electrochemical responses at the coated porous electrode.^{23,33,43-47} Surface modification with nanomaterials changes the mass transport regime to a combination of semi-infinite planar diffusion plus a finite thin layer diffusion model; failure to consider the latter has led many authors into misinterpreting the enhanced electrochemical response for signs of electrocatalysis. Henstridge *et al.*⁴⁴ performed digital simulations to support that a decrease in the oxidation potential was solely due to thin layer diffusion and there was no change in the electron transfer kinetics. Punckt *et al.*³³ studied the porosity of functionalized graphene sheets (porous drop cast *vs.* monolayer) and found that the porous electrode response dominates the apparent kinetics obtained for an irreversible redox reaction.

The assumption made during most studies that drop cast materials onto electrode surfaces is that the coating procedure completely covers the electrode such that the base material does not contribute to the electrochemical response. However, the results reported in Section 6.1.2 have suggested otherwise. Since the drop cast layers are porous in nature, it is conceivable that diffusion of the analyte within the electroactive pores all the way through to the base material may still be occurring, but the extent to which this occurs has not been quantified. Therefore, the aim of this chapter is to examine the amount of film coatings required *via* drop casting in order to ensure a complete coverage over the underlying electrode surface.

One primary difficulty in differentiating the electrochemical response of the drop cast material from that of the base electrode is that the base electrode is usually itself inert carbon based (such as glassy carbon or a form of graphite). The approach adopted in this chapter is to use copper metal as the base material which is a less chemically inert conductive material. Since copper can be electrochemically oxidized in the presence of corrosive anions such as sulfate, it is possible to determine whether the drop casting

procedure is adequately coating the electrode, since a large corrosive current will be obtained during potential scanning experiments if any of the base electrode is exposed to solution phase electrolyte ions. The electrochemical experiments described in this chapter were complimented with SEM and EDX analysis of the coated and uncoated electrode surfaces in order to determine the morphology and chemical composition of the surfaces before and after the voltammetric scanning experiments.

6.2 Results and Discussion

6.2.1 *Voltammetric behavior of Cu electrodes modified with porous layers of MWCNTs*

MWCNTs were dispersed in DMF (1 mg per 1 mL) and progressively built up on the Cu electrodes by repeatedly coating the electrode with 0 – 10 0.5 μL droplets of the MWCNT suspension as illustrated in Figure 6.3.

Figure 6.3. Images of bare copper electrode (left), copper electrode coated with 1 droplet (middle) and 5 droplets of MWCNT casting solution (right).

Sodium sulfate solution was used to obtain a clear voltammetric measure of electrochemical oxidation at the Cu electrode. The sulfate anion is known to be very aggressive to voltammetrically induced copper dissolution, which leads to the formation of deep pits and reduces passivation events.⁴⁸ Although copper is different from the

carbon materials that are usually used for the base electrode, for the purposes of this study it is assumed that attachment of the MWCNTs occurs in a similar manner. This is a reasonable assumption as the drop casting procedure of CNTs (or most other carbon nanomaterials) does not produce a covalently bonded attachment, but one where the material sticks through weak physisorption interactions. Furthermore, there was no evidence that the MWCNTs detached from the copper electrode when placed in an electrolyte solution after the drop casting procedure.

Linear sweep voltammograms obtained at a scan rate of 2 mV s^{-1} using 1 mm copper wire electrodes modified with 1, 2, 5 and 10 droplets of $0.5 \text{ }\mu\text{L}$ volume of the MWCNT drop casting solution in 0.1 M aqueous Na_2SO_4 solutions are displayed in Figure 6.4 and stacked cyclic voltammograms are displayed in Figure 6.5. The linear sweep voltammograms recorded for the complete range of the electrodes modified with 0 – 10 $0.5 \text{ }\mu\text{L}$ aliquots of MWCNT drop casting solution are provided in Figure 6.6.

The LSVs obtained at the bare Cu electrode show smooth gradual increases in the current density (j) as the potential was cycled to more positive potentials, until passivation of the electrode was observed which was represented by a sharp decrease in the voltammetric current density at $\sim +1.0 \text{ V vs. Ag/AgCl}$ (Figure 6.4, black lines). Passivation occurs due to an extensive build-up of corrosion products on the electrode surface which reduces dissolution of the Cu, although at higher potentials, the current partially recovered suggesting further dissolution of the metal. Some irregular sharp peaks can be observed during the positive potential scans which are suggestive of micro-scale pitting events occurring during the electrochemical oxidation process. The current densities were very high, indicating extensive dissolution of the Cu electrode. The electrode size was kept relatively small (1 mm) to reduce the effects of uncompensated solution resistance altering the shapes of the voltammograms.

Each LSV experiment was repeated three times using three freshly prepared and polished electrodes. For these types of corrosion experiments, the onset of the initial passivation typically occurs at variable potentials over several hundred millivolts because of the randomness of the individual micropitting events, and it has been shown that the dissolution largely proceeds by two electrons *via* the oxidation of Cu^0 to Cu^{2+} .⁴⁸

Figure 6.4. Triplicate linear sweep voltammograms (commencing at -0.5 V vs. Ag/AgCl) obtained at a scan rate of 2 mV s^{-1} at 1 mm diameter copper wire electrodes in $0.1 \text{ M Na}_2\text{SO}_4$ solutions. Black line represents bare Cu electrode and colored lines represent Cu electrode coated with porous layers of MWCNTs from the drop casting solution. (Blue lines) 1 droplet, (Red lines) 2 droplets, (Green lines) 5 droplets, (Grey lines) 10 droplets.

Figure 6.5. Triplicate cyclic voltammograms (commencing at $-0.5 \text{ V vs. Ag/AgCl}$) obtained at a scan rate of 2 mV s^{-1} at 1 mm diameter copper wire electrodes coated with porous layers of MWCNTs from the drop casting solution in $0.1 \text{ M Na}_2\text{SO}_4$ solutions. (—) Forward scan. (···) Reverse scan.

Figure 6.6. Triplex linear sweep voltammograms (commencing at -0.5 V vs. Ag/AgCl) obtained at a scan rate of 2 mV s^{-1} at 1 mm Cu electrodes coated with 1 – 10 droplets of MWCNT casting solution in $0.1 \text{ M Na}_2\text{SO}_4$.

Similar voltammetric responses were observed for Cu electrodes modified with different number of droplets of the MWCNT coating solution (Figure 6.4, colored lines) although much smaller oxidation peaks were obtained for electrodes modified with 10 droplets indicating that very high loadings of CNTs were reducing the corrosion processes. The decrease in corrosion currents at very high loadings could be due to either the reduced access of the SO_4^{2-} anions to the Cu surface, or due to the reduced ability of corrosion products to be transported away from the electrode surface thereby supporting electrode passivation. The peak maximum at which the oxidation of copper took place typically occurred between $E_p^{\text{ox}} \sim +0.7$ and $+1.3$ V vs. Ag/AgCl.

Although a decrease in corrosion current was observed as more droplets of the MWCNT coating solution were added to the electrode surface, a situation was not reached where the electrode was completely prevented from corroding, indicating that the copper surface remains in electrical contact with the solution. The average peak corrosion value for the bare copper electrode was recorded as 320 mA cm^{-2} and this decreased to 300 mA cm^{-2} for Cu electrode modified with 1 droplet of the coating solution (equivalent to $64 \mu\text{g/cm}^2$ of MWCNTs per unit area), 270 mA cm^{-2} for 2 droplets, 40 mA cm^{-2} for 5 droplets and 10 mA cm^{-2} for 10 droplets (Figure 6.5). The onset of the corrosion currents did appear to shift towards more positive potentials as more droplets were placed onto the electrode surface. However, it is difficult to ascertain whether this is due to the randomness of the dissolution events or due to kinetic factors (such as reducing rates of diffusion of SO_4^{2-} anions to the electrode surface).

Figure 6.7. Linear sweep voltammograms (commencing at -0.5 V vs. Ag/AgCl) obtained at a scan rate of 2 mV s^{-1} at 1 mm diameter copper wire electrodes in $0.1 \text{ M Na}_2\text{SO}_4$ solutions. The black line is from a bare glassy carbon electrode and the colored lines are from triplicate measurements of fresh Cu electrodes coated with 10 droplets of the MWCNT casting solution.

The linear sweep voltammogram obtained at a scan rate of 2 mV s^{-1} using a 1 mm GC electrode was plotted alongside that of a Cu wire modified with 10 droplets of the MWCNT casting solution for a clear comparison and to eliminate potential background interferences (Figure 6.7). The oxidative peaks recorded for MWCNT-modified (10 droplets) Cu electrode were considerably larger compared to the essentially flat LSV trace recorded for the GC electrode. The onset of the corrosion current varied substantially in potential at the 3 coated electrodes indicating that the coating procedure was not producing identical films. Figure 6.7 clearly shows that the underlying Cu electrode was still being electrochemically oxidized with peak currents in the range of $2.5 - 17 \text{ mA cm}^{-2}$ (which increased substantially at potentials $> +1.0 \text{ V vs. Ag/AgCl}$), suggesting that a relatively large MWCNTs loading of $640 \mu\text{g/cm}^2$ (10 droplets) is still insufficient in providing perfect coverage over the underlying copper electrode surface, even though much lesser amounts have been used in literature reports. Most studies that include

information on the carbon nanotubes loadings are typically reported in the range of 50 – 350 $\mu\text{g}/\text{cm}^2$ and the casting usually involved only a single droplet.^{23,24,34,43-46} The objective to achieve a perfect coverage over the underlying electrode was not possible even with a very large loading of MWCNTs.

6.2.2 *SEM-EDX analyses of Cu electrodes modified with porous layers of MWCNTs*

Morphological analysis of the Cu electrodes was performed so as to have a better understanding of the coverage of the carbon nanotubes formed during drop casting. While it may be a usual practice to use glassy carbon as the base electrode for carbon nanotubes casting, it is difficult to differentiate between the underlying glassy carbon electrode surface and the carbon nanotubes during elemental analysis with SEM-EDX spectroscopy. In this study, the copper electrode allowed a straightforward comparison in identifying the underlying surface among the porous layers of CNTs. Scanning electron micrographs taken before and after electrochemical oxidation at different magnifications of $\times 370$ to $\times 50,000$ are shown in Figures 6.8 – 6.16(a) and (b). An elemental characterization was performed on 0.3% of the electrode surface area ($7.85 \times 10^{-3} \text{ cm}^2$) and the composition in atomic % is displayed in Table 6.1.

The SEM image of a bare Cu electrode showed a polished surface that is represented as a lighter color compared to carbon nanotubes. The SEM image for the Cu electrode modified with one droplet of the MWCNT casting solution obtained at magnifications of $\times 370$, $\times 40,000$ and $\times 50,000$ (Figures 6.8a, 6.15a, 6.16a respectively) showed a sparse and random distribution of carbon nanotubes, which left portions of the Cu electrode surface uncovered. As the loadings increased from 64 $\mu\text{g}/\text{cm}^2$ (1 droplet) to 640 $\mu\text{g}/\text{cm}^2$ (10 droplets), the MWCNT films appeared more homogenous over the

electrode surface. The percentage of Cu detected also decreased with increasing thickness of the MWCNT coating as shown in Table 6.1.

There was a sharp drop in Cu detected when using EDX between the bare Cu and Cu with one droplet of the casting solution by 4.5 times (from 72% to 16%), whereas with 10 droplets of the coating solution, Cu was not detected which was most likely due to the heavy MWCNT loadings. A red colored crystalline material was sighted on top of the bare copper wire after oxidation and removal from the electrochemical cell. The SEM image of the bare Cu electrode after oxidation showed evidence of some crystalline material and the results from the elemental characterization indicated high values of oxygen and copper and some sulfur after electrochemical oxidation of the electrodes with 1 droplet of the casting solution, which is suggestive of the precipitation of copper oxide and copper sulfate films. An earlier study using Raman microspectroscopy had confirmed Cu_2O as the identity for the red crystalline material.⁴⁸ A significant percentage of Ni detected was likely present from the manufacturing process of the CNTs.^{34,49} Carbon is detected on the bare Cu surface prior to drop casting the CNTs due to the adsorption of adventitious atmospheric particles. A table of the metal content of the CNTs measured by inductively coupled plasma – mass spectrometry is provided in Table 6.2.

Figure 6.8. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 370$.

Figure 6.9. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 1,000$.

Figure 6.10. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 2,000$.

Figure 6.11. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 4,000$.

Figure 6.12. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 5,000$.

Figure 6.13. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 10,000$.

Figure 6.14. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 20,000$.

Figure 6.15. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 40,000$.

Figure 6.16. Scanning electron micrographs of bare copper electrodes and copper electrodes coated with 1, 5 and 10 droplets of the MWCNT casting solution taken (a) before and (b) after applying oxidative potentials (-0.5 V to $+1.5$ V vs. Ag/AgCl) at magnifications of $\times 50,000$.

Table 6.1. SEM-EDX elemental characterization at 5 kV of the bare and MWCNT-cast copper electrode surfaces before and after applying oxidative potential.

No. of drops of MWCNT casting solution		Elemental Composition (atomic %)							
		%C	%O	%Cu	%Na	%S	%Si	%F	%Ni
Before oxidation	0	24.30	4.08	71.62	-	-	-	-	-
Before oxidation	1	83.59	-	16.41	-	-	-	-	-
Before oxidation	5	97.10	1.20	0.42	-	0.13	0.09	0.54	0.51
Before oxidation	10	95.13	1.07	-	-	-	0.15	3.15	0.50
After oxidation	0	4.39	46.46	22.90	17.46	8.78	-	-	-
After oxidation	1	60.11	16.36	21.44	-	1.75	0.35	-	-
After oxidation	5	97.26	1.35	-	-	0.18	-	0.75	0.45
After oxidation	10	96.56	0.88	-	-	-	0.10	1.97	0.48

Table 6.2. Residual metal content of MWCNT analyzed by ICP-MS. Data given to 2 significant figures except for values $\leq 0.09 \mu\text{g/g}$ which are given to 1 significant figure. In each case the %RSD is $\leq 20\%$. <DL = less than detection limit.

Element	Concentration ($\mu\text{g/g}$)	Element	Concentration ($\mu\text{g/g}$)
Sc	22	B	6.1
Ti	3.8×10^2	Na	3.0×10^2
V	0.23	Mg	78
Cr	16	Al	39
Mn	2.4	K	1.3×10^2
Fe	73	Ca	1.9×10^5
Ni	2.6×10^3	Ga	4.0
Co	3.2	Ge	0.02
Cu	2.6	As	1.9
Zn	2.0×10^2	Se	0.83
Y	2.2	Rb	0.24
Zr	0.77	Sr	35
Mo	1.6	In	0.03
Ru	0.02	Sn	0.60
Rh	0.04	Sb	8.8
Pd	0.88	Te	0.16
Ag	<DL	Cs	0.01
Cd	0.19	Ba	28
La	1.1×10^2	Ce	1.7
Hf	0.51	Pr	0.01
Ta	0.31	Nd	0.03
W	0.42	Sm	0.01
Re	<DL	Eu	0.01
Os	0.04	Gd	0.01
Ir	0.07	Tb	<DL
Pt	2.3	Dy	<DL
Au	5.5	Ho	<DL
Hg	1.0	Er	<DL
Li	<DL	Tm	0.04
Be	0.03	Yb	2.4

It is clear that a single droplet of the casting MWCNT/DMF solution provides only partial coverage over the electrode, as seen in the SEM images and this supports the findings concluded earlier from the LSV experiments where a large oxidative current was recorded. The incomplete coverage allowed the solution to come into direct contact with

the copper electrode and precipitation of corrosion products occurred at the metal/electrolyte interface. It is noted that findings on the electrochemical oxidation in Section 6.2.1 did not apparently match the results from SEM-EDX analysis for 10 droplets of the casting solution. SEM-EDX demonstrated a complete coverage of MWCNTs over the underlying copper electrode and the absence of detectable copper after modification with 10 droplets ($640 \mu\text{g}/\text{cm}^2$ CNTs). However, copper was not completely protected from corrosion as shown by the relatively large oxidative currents detected during LSV experiments (Figure 6.7) despite the good coverage observed by the SEM-EDX measurements.

According to the *Kanaya-Okayama* range equation,⁵⁰ the actual penetration depth R of the primary electron during SEM measurements depends on the acceleration voltage applied and the element of the sample,

$$R = 2.76 \times 10^{-11} A E_0^{5/3} / (Z^{8/9} \times \rho) \text{ cm} \quad (6.1)$$

where E_0 is the incident electron energy in eV, A is the average atomic weight in g, ρ is the density in g/cm^3 , and Z is the atomic number of the element. One likely explanation for the lack of detection of copper is that the maximum penetration depth of the electrode surface for copper analysis is $\sim 140 \text{ nm}$ and $\sim 440 \text{ nm}$ for carbon analysis (calculated based on an acceleration voltage of 5 kV). A loading of $640 \mu\text{g}/\text{cm}^2$ reaches a thickness that extends beyond 440 nm depth and results in solely the measurement of the porous layer of carbon nanotubes. It is also possible that any copper corrosion products produced during electrochemical oxidation remain trapped inside the MWCNTs and were not detected due to the limitation of the electron penetration depth. The major corrosion product produced by electrochemically induced dissolution of Cu metal in sulfate solutions is the water soluble CuSO_4 .⁴⁸ However, as the pit depth increases, the likelihood

of other less soluble (in neutral conditions) species such as CuO or Cu₂O increases due to the localized concentration of sulfate being depleted.

6.2.3 Voltammetric behavior of GC electrodes modified with porous layers of MWCNTs

Figure 6.17. Cyclic voltammograms of (a) 0.1 M K₃Fe(CN)₆/K₄Fe(CN)₆·H₂O and (b) 0.3 M K₃Fe(CN)₆/K₄Fe(CN)₆·H₂O recorded at a scan rate of 0.1 V s⁻¹ in 0.1 M Na₂SO₄ solutions. (—) 1 mm Cu wire electrode. (---) 1 mm Pt electrode. (...) 1 mm GC electrode.

While it was thought that solution phase potassium ferri-/ferrocyanide could serve as a useful redox probe to test the condition of the electrode, it was found that the large oxidative current caused by copper corroding completely masked any solution phase

redox reactions even with the highest CNT loadings ($640 \mu\text{g}/\text{cm}^2$). Furthermore, the redox peaks of potassium ferri-/ferrocyanide were not observable at the copper electrodes even when 0.3 M of the redox analyte was added to the solution as shown in Figure 6.17.

Instead, the electrochemical behavior of potassium ferri-/ferrocyanide was investigated at a 1 mm diameter GC electrode (Figure 6.18, black line) that was modified with porous layers of MWCNTs (Figure 6.18, coloured lines) in 0.1 M aqueous Na_2SO_4 solutions. The voltammetric responses recorded at the modified MWCNT-coated GC electrode had a comparable peak-to-peak separation but up to a three-fold increase in the voltammetric peak currents as compared to responses at a bare glassy carbon. Other studies have suggested that the increase in the peak current was due to an increase in the electroactive surface area *via* casting porous layers of MWCNTs onto the electrode. It was also proposed that a thin film diffusion-related effect must be taken into account during the interpretation of the electrochemical signal at the porous electrode.^{23,33,43-47} However, experiments using copper electrodes in this work have also indicated that diffusion can occur all the way to the base electrode surface, especially when relatively low CNT loadings are used corresponding to 1 – 5 droplets of the coating solution ($< 320 \mu\text{g}/\text{cm}^2$ CNTs). Surface modification by adding layers of MWCNTs changes the diffusion regime from a classical semi-infinite planar model to a combination of semi-infinite diffusion (from the bulk solution to the electrode) and a finite thin layer diffusion (molecules accumulated and are trapped in pockets of solution between the porous nanotube layers) model.

There were some observable differences in the peak currents as the number of droplets of casting solution was varied, as shown in the CV results in Figure 6.18. However, the variations of the recorded peak currents with multiple droplets of casting solution are likely caused by small variations in reproducibility in the drop casting procedure (as seen in the data in Figure 6.7 when the Cu electrode was coated). Hence,

there is not a notable improvement in the peak currents when increasing the number of drop-coated layers beyond 2.

Figure 6.18. Cyclic voltammograms of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ recorded at a scan rate of 0.1 V s^{-1} at a 1 mm diameter glassy carbon electrode in 0.1 M Na_2SO_4 solutions. Black line represents bare glassy carbon electrode and colored lines represent the glassy carbon electrode coated with (blue line) 2 droplets, (green line) 4 droplets, (brown line) 8 droplets, (red line) 10 droplets of the MWCNT casting solution.

6.2.4 Voltammetric behavior of GC electrodes modified with nano-sized alumina powder

One issue with interpreting the results of the copper coating experiments is that the corrosion current is much greater than the diffusion current that normally originates when mM amounts of analyte diffuse to the electrode surface. Therefore, it is possible that the corrosive currents that are observed at very high CNT loadings (Figure 6.7) originate from a small number of discrete pin holes in the surface coverage allowing SO_4^{2-} anions to reach the electrode surface, rather than diffusion of SO_4^{2-} through the outer nanofibrous CNT layers. However, it is likely that if there were many pin holes on

the surface, then there would be only a small change in the corrosion current as the CNTs layers were deposited, as it has been shown that diffusion currents measured at blocked surfaces with a large number of microscopic active sites can be very high.⁵¹ Pin holes are difficult to detect from SEM images and the current response that is observed during voltammetric scanning is representative of an average over the entire electrode surface, and does not allow the identification of individual electroactive sites.

Therefore, drop casting experiments on GC electrodes were also performed using the nanomaterial Al_2O_3 (300 nm particle size), which is an electrical insulator that cannot itself contribute to the diffusion current. It was reasoned that if the drop casting procedure was allowing a small number of random pin holes in the nanoparticle coating, then a diffusion response for the potassium ferri-/ferrocyanide would still be obtained, but the current values would be considerably smaller than obtained for diffusion to the exposed bare electrode. Conversely, if the diffusion currents did not change with added Al_2O_3 nanoparticles, it would imply that the drop casting procedure was producing highly porous layers allowing good access to the base electrode.

The drop casting of Al_2O_3 nanoparticles onto the GC electrode (Figure 6.19) did not result in an enhancement in the voltammetric responses for potassium ferri-/ferrocyanide as was observed in the presence of the conducting MWCNTs (Figure 6.18). Instead, the peak currents decreased only slightly and the anodic to cathodic peak separation increased as more droplets of the Al_2O_3 drop casting solution were added. The results from the Al_2O_3 experiments clearly indicate that the ferri-/ferrocyanide were able to diffuse to the base electrode surface despite the nanomaterial coating and the small drop in peak current suggest that the diffusion is only mildly hindered.

Figure 6.19. Cyclic voltammograms of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ recorded at a scan rate of 0.1 V s^{-1} at a 1 mm diameter glassy carbon electrode in 0.1 M Na_2SO_4 solutions. Black line represents bare glassy carbon electrode and colored lines represent the glassy carbon electrode coated with (blue line) 2 droplets, (green line) 4 droplets, (brown line) 8 droplets, (red line) 10 droplets of the alumina powder casting solution.

Therefore, the results from the combined Al_2O_3 and MWCNT casting experiments indicate that it is likely that the drop casting procedure does not prevent access of the electroactive molecules to the base electrode material. Nevertheless, when comparing the data in Figures 6.18 and 6.19, it is not possible to make conclusions regarding whether the increased voltammetric peak currents originate from thin-layer behavior or simply due to an increase in electrode surface area, because the Al_2O_3 and CNT materials themselves have different nanoporous properties.

6.3 Conclusions

In recent years, modifying electrodes with nanoscale carbonaceous materials has been proposed as a method for enhancing their performance for many applications varying from electrochemical detection, electrochemical biosensing to energy storage in batteries. This chapter provides evidence of electrochemical reactions occurring at the underlying base electrode of MWCNT-modified electrodes even after the application of 10 coating droplets, which was equivalent to a heavy loading of $640\text{ }\mu\text{g}/\text{cm}^2$ of MWCNTs per unit area of the electrode. It was demonstrated that the solution phase analyte and electrolyte molecules are able to diffuse through the electroactive CNT pores. Increasing the loading of the CNTs did alter the degree of diffusion of the solution phase analyte to the base electrode but it was not possible to completely stop reactions occurring at the underlying copper electrode using the drop casting method. Therefore, studies that have relied solely on the anodic-to-cathodic peak to peak separations during CV measurements as a method for measuring heterogeneous electron transfer rates of coated nanomaterials are open to reinterpretation, as the base material is also contributing to the current-potential trace. Although this study was restricted to MWCNTs and Al_2O_3 , it is likely that other porous nanomaterials (i.e. chemically modified graphenes) would also allow diffusion of analyte molecules through the porous structure to the electrode support.

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Chapter 7

Summary and Conclusion

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Mechanistic studies on compounds that undergo metabolic reaction are difficult to perform in real biological systems. However, investigations with electrochemical methods can provide useful information on potential metabolic pathways since the reactions can be performed on the compounds separately and in the absence of a complicated biological matrix, reducing the presence of complex interfering species. The metabolic pathways leading to potentially toxic metabolites in lipophilic systems are mostly dominated by oxidation processes which can be studied with electrochemical oxidation.

In this thesis, the electrochemical oxidation behavior of two types of artificial hydroxyl containing emerging contaminants namely bisphenol A and phenolic benzotriazoles and the natural hydroxyl containing vitamin D compounds were examined in aprotic solvents using a range of electrochemical methods. The oxidative mechanism for the electrochemical oxidation of the compounds including the exact electron transfer (E) and chemical steps (C) as well as the identity of the intermediate species and final oxidized products are proposed, which highlight the importance of the intermediate species as reactive metabolites during the oxidative degradation pathway in biological systems and provide useful insights into the reactions that can potentially occur in lipophilic environments. The electrochemical oxidation of the two artificial phenolic compounds occurred at the phenolic moiety in a mechanism that is similar to phenols in general. The electrochemical oxidation of the natural hydroxyl containing vitamin D compounds likely occurred at the triene moiety.

In Chapter 3, BPA undergoes a chemically irreversible oxidation process at $\sim +0.9$ V vs. Fc/Fc^+ in CH_3CN to form compounds that cannot be electrochemically converted back to the starting material on the short voltammetric timescale ($\leq$ seconds). To overcome the effects of electrode fouling during controlled potential electrolysis experiments, NO^+ was used as a one-electron chemical oxidant. It is proposed that BPA can be oxidized *via* a four-electron/two-proton process ($-4\text{e}^-/-2\text{H}^+$) in a $2 \times \text{ECE}$

mechanism to form a relatively unstable dication which reacts quickly in the presence of water in acetonitrile. A new oxidized product hydroxylated bisdienone was isolated from the chemical oxidation of BPA with 4 mol equiv of $\text{NO}^+\text{SbF}_6^-$ in low water content CH_3CN . However, as the water content of the solvent increased, it was found that the chemical oxidation mechanism produced a nitration product in high yield.

In Chapter 4, the electrochemical behavior of selected phenolic benzotriazoles, namely UV234 and UV327, were examined and cyclic voltammetry showed that both phenolic BZTs undergo a chemically irreversible oxidation process at $\sim +1.0$ V *vs.* Fc/Fc^+ on the short voltammetric timescale ($\leq$ seconds). UV234 and UV327 displayed similar behavior and can be electrochemically oxidized *via* a two-electron/one-proton process ($-2e^-/-1H^+$) in an ECE mechanism under neutral and acidic conditions. In basic conditions, cyclic voltammetry experiments indicated that the corresponding phenolates (prepared by reacting the phenols with equiv. molar of *n*-Bu₄NOH) were oxidized at $E_p^{\text{ox}} \sim -0.2$ V *vs.* Fc/Fc^+ *via* a one-electron diffusion controlled process with $(i_p^{\text{ox}}/i_p^{\text{red}}) \gg 1$, suggesting that the produced phenoxyl radicals decomposed rapidly *via* a chemical step. However, EPR experiments performed on the bulk electrolyzed solutions of the phenolates after one-electron oxidation indicated long lifetimes of the UV234[•] and UV327[•] phenoxyl radicals (constant signal intensities were measured over 3 h). Long timescale controlled potential electrolysis experiments combined with spectroscopic (UV-vis and EPR) studies provided evidence of a reversible dimerization mechanism between the phenoxyl radicals forming dimers. The overall mechanism of phenolic benzotriazoles in aprotic organic solvents containing base is a CEC_{dim} process; phenolic BZTs initially deprotonate (C step) to form the benzotriazolyl-substituted phenolates which oxidized *via* one-electron (E step) to give neutral benzotriazolyl-substituted phenoxyl radicals that immediately undergo a reversible dimerization (C_{dim} -step) to form a dimer which exists in equilibrium with the radicals. A comparison of the relative EPR signal intensities of the

phenoxyl radicals with the long-lived signal of $\text{TCNQ}^{\bullet-}$ allowed an estimation of the equilibrium constant of the dimerization reaction (K_{eq}) of 10^5 to 10^6 L mol^{-1} . Despite the seemingly high equilibrium constant in favour of dimer formation, the rapid rates of reactions between the phenoxyl radicals and their dimeric forms enabled the radicals to be completely electrochemically converted back to the phenolate forms on the electrolysis time scale.

In Chapter 5, the electrochemical behavior of vitamins D_3 and D_2 were examined and cyclic voltammetry showed that both vitamins were electrochemically oxidized at $E_{\text{p}}^{\text{ox}} \sim +0.8 \text{ V vs. Fc/Fc}^+$ *via* a one-electron chemically irreversible process on the short voltammetric timescale ($\leq$ seconds). Varying the scan rate and temperature did not strongly affect the voltammetric signals recorded on platinum and glassy carbon electrode surfaces, with the oxidation process remaining chemically irreversible over the range of scan rates and temperatures tested, suggesting that the initially formed cation radical was not long-lived. Repetitive CV experiments indicated that the oxidized product partially adsorbed onto the electrode surface, resulting in diminishing peak currents with multiple scans. Bulk controlled potential electrolysis of the vitamin D compounds performed by alternating several cycles of oxidative electrolysis and reductive pulsed stripping proved to be effective in stripping the adsorbed species off the electrode surfaces. Longer time scale bulk electrolysis experiments led to the detection of a new oxidation peak appearing at less positive potentials as the electrolysis progressed, suggesting that the compounds underwent oxidation on long timescales (minutes to hours) *via* a two electron process. The vitamins were most likely initially oxidized *via* a one-electron (E-step) to form cation radicals, which reacted homogeneously in two chemical steps (C-steps) where one chemical step was fast ($< 1 \text{ s}$) and one was relatively slow ($> 1 \text{ s}$). On the electrolysis timescale, the oxidized product then undergoes a second electron transfer (E) at less positive potentials, so the overall mechanism is an ECCE process. Chemical oxidation of

vitamin D₃ with 2 mol equiv of NO⁺SbF₆⁻ in acetonitrile/dichloromethane 1:4 ratio (v/v) resulted in the complete oxidation of the starting material in an overall two-electron process, with NMR analysis of the reaction mixture indicating that the triene moiety is absent from the products and this essentially highlights the possibility of an oxidative route other than the widely known hydroxylation reactions.

For all the three types of compounds, electrode fouling was encountered at the bare electrode surfaces during electrochemical oxidation, especially with BPA and vitamin D. The adsorption processes resulted in diminishing oxidative peak currents with repetitive scanning. Investigation at a MWCNT-coated GC electrode suggested that electrode modification with nanomaterials was not helpful in resolving the effects of electrode fouling.

In Chapter 6, we assessed the surface coverage provided by the nanomaterials drop cast onto electrodes and if the nanomaterial coatings were adequately covering the electrode such that the base material does not contribute to the electrochemical response. The electrochemical responses recorded at 1 mm Cu electrodes modified with porous layers of drop cast MWCNTs in 0.1 M Na₂SO₄ aqueous solutions were examined. Standard amounts of the MWCNTs that are typically used for the drop casting procedure were deposited drop wise on the surface of the copper electrodes. The layers were progressively built up on the electrode surface by varying the number of droplets from 0 to 10. The ability of the MWCNTs to cover and prevent diffusion to the base copper electrode was assessed by performing oxidative cyclic and linear sweep voltammetry experiments in the presence of aggressive SO₄²⁻ supporting electrolyte ions, where a large oxidative current indicated the occurrence of corroding Cu metal. It was demonstrated that a total of 10 droplets of the coating solution (equivalent to 640 µg/cm² of MWCNTs per unit area) was insufficient in providing a complete coverage over the underlying electrode surface even though considerably lesser CNTs loadings have been applied in the

majority of literature reports. The results indicate that the drop casting procedure does not prevent access of solution-phase electroactive molecules from diffusing through the nanomaterial coating to the base electrode. Therefore, it cannot be assumed that the base electrode, nor the pore structure of the coating material, does not significantly contribute to the observed voltammetric response. The "drop casting" method for the suspension of nanomaterials on conducting surfaces is a commonly used procedure for evaluating the cast materials electrochemical properties and in this chapter, we pinpoint a key limitation of the method and recommend that the studies that utilize nanomaterial coatings on electrode surfaces need to take into account the effect of the base electrode on the overall measured current or potential.

Although metabolites generated with electrochemical oxidation have been shown to be similar to the metabolites generated from enzymatic oxidation, not all metabolic pathways can be mimicked by electrochemical oxidation as there can be mechanistic differences between the two methods due to the limitation of studying the compounds in the same exact conditions present in real biological systems. While it is still not possible for electrochemical oxidation to replace conventional metabolism studies in *in-vitro* and *in-vivo* systems in the investigation of their potential toxicity at this point, electrochemical oxidation provides valuable information on the reaction intermediates and oxidative pathways which can be used for future study that couples electrochemical oxidation to LC/MS. Along with the incorporation of phase II conjugative enzymes, EC coupled to LC/MS can be used to simulate oxidative metabolism and propose detailed metabolic pathways that lead to the complete range of detected oxidized products.

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Appendix

Chapter 3

*Characterization data ^1H , ^{13}C and DEPT NMR, HRMS, FTIR spectroscopy and UV-vis spectroscopy of compounds **1** – **3** are provided in this section along with the X-ray crystal structure data of compound **3**.*

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Chapter 3

Figure A.3.1. ^1H NMR spectrum of **1** (CDCl_3 , 400 MHz).

Figure A.3.2. ^{13}C NMR spectrum of **1** (CDCl_3 , 400 MHz).

YaYun-001-1 Dept135 AV400 CDCl₃

Figure A.3.3. DEPT NMR spectrum of **1** (CDCl₃, 400 MHz).

¹H NMR, BBFO1

Figure A.3.4. ¹H NMR spectrum of **2** (CDCl₃, 400 MHz).

¹³C NMR, BBFO1

Figure A.3.5. ¹³C NMR spectrum of **2** (CDCl₃, 400 MHz).

Dept 135 NMR, BBFO1

Figure A.3.6. DEPT NMR spectrum of **2** (CDCl₃, 400 MHz).

^1H , AV400MHz, CDCl_3

Figure A.3.7. ^1H NMR spectrum of **3** (CDCl_3 , 400 MHz).

^{13}C , AV400MHz, CDCl_3

Figure A.3.8. ^{13}C NMR spectrum of **3** (CDCl_3 , 400 MHz).

¹³S Dept, AV400MHz, CDCl₃

Figure A.3.9. DEPT NMR spectrum of **3** (CDCl₃, 400 MHz).

Figure A.3.10. UV-vis absorption spectrum of **3**.

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

32 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-34 H: 0-31 O: 3-5 P: 0-3

C15H16O4

YY-001-1 33 (0.747)

1: TOF MS ES+
1.01e+000

Minimum:								
Maximum:	5.0	5.0	-1.5	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
261.1131	261.1127	0.4	1.5	7.5	13.3	0.0	C15	H17 O4

Figure A.3.11. HRMS spectrum of 1.

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

19 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-16 H: 0-20 N: 0-2 O: 0-6

C15H14N2O6

YY-001-21 (0.469)

1: TOF MS ES+
1.01e+000

Minimum:								
Maximum:	5.0	10.0	-1.5	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
319.0929	319.0930	-0.1	-0.3	9.5	12.7	0.0	C15	H15 N2 O6

Figure A.3.12. HRMS spectrum of 3.

Figure A.3.13. FTIR spectrum of **1**.

Figure A.3.14. FTIR spectrum of **3**.

(a)

(b)

Figure A.3.15. X-ray crystallographic structures (a) and (b) for **3**.

Table A.3.I. Crystal data and structure refinement for **3**.

Identification code	3	
Empirical formula	C ₁₅ H ₁₄ N ₂ O ₆	
Formula weight	318.28	
Temperature	103(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 8.3972(7) Å	a = 66.993(5)°.
	b = 12.5662(11) Å	b = 76.615(4)°.
	c = 15.3616(12) Å	g = 77.866(5)°.
Volume	1438.5(2) Å ³	
Z	4	
Density (calculated)	1.470 Mg/m ³	
Absorption coefficient	0.116 mm ⁻¹	
F(000)	664	
Crystal size	0.40 x 0.14 x 0.10 mm ³	
Theta range for data collection	1.78 to 26.43°.	
Index ranges	-8<= <i>h</i> <=10, -15<= <i>k</i> <=15, -19<= <i>l</i> <=19	
Reflections collected	16205	
Independent reflections	5832 [R(int) = 0.0544]	
Completeness to theta = 26.43°	98.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9885 and 0.9552	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	5832 / 0 / 423	
Goodness-of-fit on F²	1.029	
Final R indices [I>2sigma(I)]	R1 = 0.0522, wR2 = 0.1271	
R indices (all data)	R1 = 0.0872, wR2 = 0.1587	
Largest diff. peak and hole	0.325 and -0.307 e.Å ⁻³	

Table A.3.2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **3**. $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	3387(3)	-238(2)	2505(2)	16(1)
C(2)	3738(3)	-493(2)	3416(2)	16(1)
C(3)	2499(3)	-671(2)	4220(2)	19(1)
C(4)	885(3)	-608(2)	4079(2)	21(1)
C(5)	550(3)	-363(2)	3188(2)	19(1)
C(6)	1798(3)	-175(2)	2373(2)	15(1)
C(7)	1319(2)	116(2)	1394(2)	15(1)
C(8)	446(3)	-868(2)	1445(2)	18(1)
C(9)	2841(3)	223(2)	592(2)	18(1)
C(10)	228(3)	1299(2)	1156(2)	14(1)
C(11)	-1336(3)	1491(2)	940(2)	15(1)
C(12)	-2238(2)	2611(2)	707(2)	15(1)
C(13)	-1606(3)	3565(2)	688(2)	15(1)
C(14)	-27(3)	3352(2)	932(2)	17(1)
C(15)	848(3)	2259(2)	1155(2)	16(1)
C(16)	5021(3)	4858(2)	7696(2)	18(1)
C(17)	5445(3)	5940(2)	7524(2)	19(1)
C(18)	4416(3)	6978(2)	7140(2)	23(1)
C(19)	2906(3)	6884(2)	6963(2)	22(1)
C(20)	2490(3)	5812(2)	7140(2)	18(1)
C(21)	3531(3)	4766(2)	7506(2)	16(1)
C(22)	2953(3)	3606(2)	7720(2)	17(1)
C(23)	1462(3)	3466(2)	8535(2)	20(1)
C(24)	4275(3)	2556(2)	8071(2)	21(1)
C(25)	2518(3)	3615(2)	6799(2)	16(1)
C(26)	947(3)	3555(2)	6709(2)	17(1)
C(27)	639(3)	3541(2)	5860(2)	18(1)
C(28)	1884(3)	3593(2)	5075(2)	21(1)
C(29)	3474(3)	3681(2)	5159(2)	22(1)
C(30)	3771(3)	3691(2)	5996(2)	20(1)
N(1)	5451(2)	-596(2)	3510(2)	21(1)
N(2)	-3878(2)	2757(2)	492(1)	21(1)
N(3)	7002(3)	5973(2)	7758(2)	29(1)
N(4)	-1039(2)	3470(2)	5817(2)	23(1)
O(1)	6525(2)	-516(2)	2806(1)	28(1)
O(2)	5776(2)	-775(2)	4311(1)	29(1)
O(3)	2705(2)	-885(2)	5116(1)	28(1)
O(4)	-4396(2)	1919(2)	489(2)	36(1)
O(5)	-4723(2)	3735(2)	324(1)	27(1)
O(6)	-2358(2)	4665(1)	465(1)	20(1)
O(7)	7831(2)	5051(2)	8146(2)	42(1)
O(8)	7447(2)	6932(2)	7577(1)	39(1)
O(9)	4781(2)	8058(2)	6935(1)	36(1)
O(10)	-2111(2)	3411(2)	6521(1)	32(1)
O(11)	-1359(2)	3470(2)	5065(1)	30(1)
O(12)	1670(2)	3577(2)	4238(1)	33(1)

Table A.3.3. Bond lengths [Å] for **3**.

Bond	Length	Bond	Length
C(1)-C(6)	1.377(3)	C(18)-C(19)	1.395(3)
C(1)-C(2)	1.396(3)	C(19)-C(20)	1.370(3)
C(1)-H(1)	0.95	C(19)-H(19)	0.95
C(2)-C(3)	1.395(3)	C(20)-C(21)	1.406(3)
C(2)-N(1)	1.452(3)	C(20)-H(20)	0.95
C(3)-O(3)	1.341(3)	C(21)-C(22)	1.523(3)
C(3)-C(4)	1.403(3)	C(22)-C(23)	1.536(3)
C(4)-C(5)	1.363(3)	C(22)-C(25)	1.537(3)
C(4)-H(4)	0.95	C(22)-C(24)	1.538(3)
C(5)-C(6)	1.410(3)	C(23)-H(23A)	0.98
C(5)-H(5)	0.95	C(23)-H(23B)	0.98
C(6)-C(7)	1.532(3)	C(23)-H(23C)	0.98
C(7)-C(10)	1.530(3)	C(24)-H(24A)	0.98
C(7)-C(8)	1.533(3)	C(24)-H(24B)	0.98
C(7)-C(9)	1.544(3)	C(24)-H(24C)	0.98
C(8)-H(8A)	0.98	C(25)-C(26)	1.378(3)
C(8)-H(8B)	0.98	C(25)-C(30)	1.410(3)
C(8)-H(8C)	0.98	C(26)-C(27)	1.395(3)
C(9)-H(9A)	0.98	C(26)-H(26)	0.95
C(9)-H(9B)	0.98	C(27)-C(28)	1.390(3)
C(9)-H(9C)	0.98	C(27)-N(4)	1.448(3)
C(10)-C(11)	1.379(3)	C(28)-O(12)	1.347(3)
C(10)-C(15)	1.409(3)	C(28)-C(29)	1.402(3)
C(11)-C(12)	1.402(3)	C(29)-C(30)	1.369(3)
C(11)-H(11)	0.95	C(29)-H(29)	0.95
C(12)-C(13)	1.397(3)	C(30)-H(30)	0.95
C(12)-N(2)	1.448(3)	N(1)-O(1)	1.223(2)
C(13)-O(6)	1.338(3)	N(1)-O(2)	1.246(2)
C(13)-C(14)	1.405(3)	N(2)-O(4)	1.223(3)
C(14)-C(15)	1.367(3)	N(2)-O(5)	1.246(2)
C(14)-H(14)	0.95	N(3)-O(7)	1.223(3)
C(15)-H(15)	0.95	N(3)-O(8)	1.244(3)
C(16)-C(21)	1.385(3)	N(4)-O(10)	1.225(2)
C(16)-C(17)	1.388(3)	N(4)-O(11)	1.245(2)
C(16)-H(16)	0.95	O(3)-H(3)	0.84
C(17)-C(18)	1.398(3)	O(6)-H(6)	0.84
C(17)-N(3)	1.447(3)	O(9)-H(9)	0.84
C(18)-O(9)	1.352(3)	O(12)-H(12)	0.84

Table A.3.4. Bond angles [°] for **3**.

Bond	Angle	Bond	Angle
C(6)-C(1)-C(2)	120.8(2)	C(19)-C(18)-C(17)	117.2(2)
C(6)-C(1)-H(1)	119.6	C(20)-C(19)-C(18)	120.5(2)
C(2)-C(1)-H(1)	119.6	C(20)-C(19)-H(19)	119.7
C(3)-C(2)-C(1)	121.59(19)	C(18)-C(19)-H(19)	119.7
C(3)-C(2)-N(1)	120.44(19)	C(19)-C(20)-C(21)	122.7(2)
C(1)-C(2)-N(1)	118.0(2)	C(19)-C(20)-H(20)	118.7
O(3)-C(3)-C(2)	126.0(2)	C(21)-C(20)-H(20)	118.7
O(3)-C(3)-C(4)	116.9(2)	C(16)-C(21)-C(20)	116.9(2)
C(2)-C(3)-C(4)	117.2(2)	C(16)-C(21)-C(22)	123.5(2)
C(5)-C(4)-C(3)	121.0(2)	C(20)-C(21)-C(22)	119.59(19)
C(5)-C(4)-H(4)	119.5	C(21)-C(22)-C(23)	107.57(18)
C(3)-C(4)-H(4)	119.5	C(21)-C(22)-C(25)	108.56(18)
C(4)-C(5)-C(6)	122.0(2)	C(23)-C(22)-C(25)	112.32(18)
C(4)-C(5)-H(5)	119	C(21)-C(22)-C(24)	112.69(19)
C(6)-C(5)-H(5)	119	C(23)-C(22)-C(24)	107.08(19)
C(1)-C(6)-C(5)	117.4(2)	C(25)-C(22)-C(24)	108.68(18)
C(1)-C(6)-C(7)	123.7(2)	C(22)-C(23)-H(23A)	109.5
C(5)-C(6)-C(7)	118.90(18)	C(22)-C(23)-H(23B)	109.5
C(10)-C(7)-C(6)	107.12(17)	H(23A)-C(23)-H(23B)	109.5
C(10)-C(7)-C(8)	113.09(17)	C(22)-C(23)-H(23C)	109.5
C(6)-C(7)-C(8)	108.86(19)	H(23A)-C(23)-H(23C)	109.5
C(10)-C(7)-C(9)	108.00(18)	H(23B)-C(23)-H(23C)	109.5
C(6)-C(7)-C(9)	112.09(17)	C(22)-C(24)-H(24A)	109.5
C(8)-C(7)-C(9)	107.75(18)	C(22)-C(24)-H(24B)	109.5
C(7)-C(8)-H(8A)	109.5	H(24A)-C(24)-H(24B)	109.5
C(7)-C(8)-H(8B)	109.5	C(22)-C(24)-H(24C)	109.5
H(8A)-C(8)-H(8B)	109.5	H(24A)-C(24)-H(24C)	109.5
C(7)-C(8)-H(8C)	109.5	H(24B)-C(24)-H(24C)	109.5
H(8A)-C(8)-H(8C)	109.5	C(26)-C(25)-C(30)	117.4(2)
H(8B)-C(8)-H(8C)	109.5	C(26)-C(25)-C(22)	123.35(19)
C(7)-C(9)-H(9A)	109.5	C(30)-C(25)-C(22)	119.24(19)
C(7)-C(9)-H(9B)	109.5	C(25)-C(26)-C(27)	120.6(2)
H(9A)-C(9)-H(9B)	109.5	C(25)-C(26)-H(26)	119.7
C(7)-C(9)-H(9C)	109.5	C(27)-C(26)-H(26)	119.7
H(9A)-C(9)-H(9C)	109.5	C(28)-C(27)-C(26)	121.9(2)
H(9B)-C(9)-H(9C)	109.5	C(28)-C(27)-N(4)	120.5(2)
C(11)-C(10)-C(15)	117.4(2)	C(26)-C(27)-N(4)	117.63(19)
C(11)-C(10)-C(7)	124.0(2)	O(12)-C(28)-C(27)	124.8(2)
C(15)-C(10)-C(7)	118.58(18)	O(12)-C(28)-C(29)	117.7(2)

(Table A.3.4. is continued on the next page)

Table A.3.4. Bond angles [°] for **3**. (Continued).

Bond	Angle	Bond	Angle
C(10)-C(11)-C(12)	120.3(2)	C(27)-C(28)-C(29)	117.5(2)
C(10)-C(11)-H(11)	119.8	C(30)-C(29)-C(28)	120.4(2)
C(12)-C(11)-H(11)	119.8	C(30)-C(29)-H(29)	119.8
C(13)-C(12)-C(11)	122.10(19)	C(28)-C(29)-H(29)	119.8
C(13)-C(12)-N(2)	120.13(19)	C(29)-C(30)-C(25)	122.2(2)
C(11)-C(12)-N(2)	117.8(2)	C(29)-C(30)-H(30)	118.9
O(6)-C(13)-C(12)	126.58(19)	C(25)-C(30)-H(30)	118.9
O(6)-C(13)-C(14)	116.4(2)	O(1)-N(1)-O(2)	122.23(18)
C(12)-C(13)-C(14)	117.0(2)	O(1)-N(1)-C(2)	119.10(18)
C(15)-C(14)-C(13)	120.6(2)	O(2)-N(1)-C(2)	118.66(19)
C(15)-C(14)-H(14)	119.7	O(4)-N(2)-O(5)	122.06(18)
C(13)-C(14)-H(14)	119.7	O(4)-N(2)-C(12)	119.30(19)
C(14)-C(15)-C(10)	122.6(2)	O(5)-N(2)-C(12)	118.64(19)
C(14)-C(15)-H(15)	118.7	O(7)-N(3)-O(8)	121.9(2)
C(10)-C(15)-H(15)	118.7	O(7)-N(3)-C(17)	118.7(2)
C(21)-C(16)-C(17)	120.7(2)	O(8)-N(3)-C(17)	119.4(2)
C(21)-C(16)-H(16)	119.6	O(10)-N(4)-O(11)	121.46(19)
C(17)-C(16)-H(16)	119.6	O(10)-N(4)-C(27)	119.32(19)
C(16)-C(17)-C(18)	122.0(2)	O(11)-N(4)-C(27)	119.22(19)
C(16)-C(17)-N(3)	117.9(2)	C(3)-O(3)-H(3)	109.5
C(18)-C(17)-N(3)	120.0(2)	C(13)-O(6)-H(6)	109.5
O(9)-C(18)-C(19)	118.1(2)	C(18)-O(9)-H(9)	109.5
O(9)-C(18)-C(17)	124.7(2)	C(28)-O(12)-H(12)	109.5

Table A.3.5. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **3**. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^*2U^{11} + \dots + 2 h k a^* b^* U^{12}]$.

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
C(1)	14(1)	14(1)	18(1)	-7(1)	-1(1)	0(1)
C(2)	13(1)	15(1)	24(1)	-7(1)	-9(1)	1(1)
C(3)	24(1)	16(1)	15(1)	-3(1)	-8(1)	-3(1)
C(4)	18(1)	25(2)	18(1)	-6(1)	0(1)	-4(1)
C(5)	14(1)	22(1)	21(1)	-7(1)	-4(1)	-3(1)
C(6)	14(1)	12(1)	18(1)	-5(1)	-6(1)	1(1)
C(7)	13(1)	18(1)	15(1)	-8(1)	-2(1)	-2(1)
C(8)	18(1)	17(1)	19(1)	-6(1)	-6(1)	-1(1)
C(9)	16(1)	23(1)	16(1)	-10(1)	-2(1)	-2(1)
C(10)	14(1)	17(1)	11(1)	-6(1)	-2(1)	-3(1)
C(11)	17(1)	14(1)	15(1)	-6(1)	-3(1)	-5(1)
C(12)	11(1)	19(1)	15(1)	-6(1)	-3(1)	-2(1)
C(13)	15(1)	18(1)	11(1)	-5(1)	0(1)	-1(1)
C(14)	17(1)	18(1)	16(1)	-7(1)	-1(1)	-7(1)
C(15)	13(1)	21(1)	15(1)	-8(1)	-1(1)	-5(1)
C(16)	16(1)	23(1)	15(1)	-10(1)	-3(1)	-1(1)
C(17)	17(1)	29(2)	18(1)	-14(1)	-1(1)	-7(1)
C(18)	29(1)	22(2)	20(1)	-11(1)	3(1)	-11(1)
C(19)	23(1)	19(1)	19(1)	-5(1)	-3(1)	0(1)
C(20)	15(1)	20(1)	18(1)	-7(1)	-4(1)	-1(1)
C(21)	15(1)	19(1)	14(1)	-7(1)	0(1)	-3(1)
C(22)	16(1)	16(1)	16(1)	-4(1)	-1(1)	-3(1)
C(23)	21(1)	23(1)	17(1)	-6(1)	-3(1)	-6(1)
C(24)	23(1)	19(1)	21(1)	-6(1)	-6(1)	0(1)
C(25)	17(1)	11(1)	17(1)	-4(1)	-4(1)	-1(1)
C(26)	17(1)	15(1)	17(1)	-5(1)	-1(1)	-3(1)
C(27)	17(1)	16(1)	20(1)	-3(1)	-6(1)	-3(1)
C(28)	26(1)	24(2)	16(1)	-8(1)	-5(1)	-5(1)
C(29)	22(1)	28(2)	18(1)	-9(1)	3(1)	-7(1)
C(30)	15(1)	20(1)	24(1)	-9(1)	-2(1)	-4(1)
N(1)	19(1)	18(1)	28(1)	-10(1)	-10(1)	0(1)
N(2)	16(1)	22(1)	27(1)	-12(1)	-8(1)	0(1)
N(3)	24(1)	47(2)	27(1)	-24(1)	-1(1)	-12(1)
N(4)	23(1)	22(1)	23(1)	-5(1)	-6(1)	-7(1)
O(1)	14(1)	42(1)	34(1)	-20(1)	-4(1)	-2(1)
O(2)	30(1)	33(1)	28(1)	-10(1)	-18(1)	-2(1)
O(3)	27(1)	42(1)	17(1)	-7(1)	-8(1)	-11(1)
O(4)	24(1)	29(1)	69(2)	-25(1)	-24(1)	0(1)
O(5)	20(1)	21(1)	42(1)	-12(1)	-14(1)	5(1)
O(6)	17(1)	16(1)	27(1)	-8(1)	-7(1)	1(1)
O(7)	25(1)	55(2)	57(1)	-28(1)	-20(1)	2(1)
O(8)	41(1)	53(1)	42(1)	-28(1)	-1(1)	-28(1)
O(9)	41(1)	26(1)	45(1)	-15(1)	-1(1)	-16(1)
O(10)	20(1)	47(1)	32(1)	-14(1)	-1(1)	-12(1)
O(11)	31(1)	37(1)	26(1)	-8(1)	-14(1)	-10(1)
O(12)	33(1)	53(1)	21(1)	-17(1)	-4(1)	-16(1)

Table A.3.6. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **3**.

	x	y	z	U(eq)
H(1)	4255	-107	1969	19
H(4)	12	-739	4612	25
H(5)	-558	-316	3115	23
H(8A)	1183	-1608	1627	27
H(8B)	159	-700	817	27
H(8C)	-563	-926	1925	27
H(9A)	3426	845	545	27
H(9B)	2482	408	-20	27
H(9C)	3581	-518	742	27
H(11)	-1806	861	948	18
H(14)	437	3973	943	20
H(15)	1915	2141	1314	19
H(16)	5760	4172	7946	21
H(19)	2159	7568	6719	26
H(20)	1455	5775	7009	21
H(23A)	1795	3467	9105	30
H(23B)	1056	2726	8686	30
H(23C)	583	4114	8333	30
H(24A)	5280	2645	7588	32
H(24B)	3860	1836	8174	32
H(24C)	4526	2519	8675	32
H(26)	66	3523	7230	20
H(29)	4350	3735	4633	27
H(30)	4859	3750	6037	23
H(3)	3705	-890	5122	42
H(6)	-3297	4703	342	30
H(9)	5718	8004	7067	54
H(12)	690	3484	4279	49

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