Electrochemical studies of vitamin A and vitamin E in organic solvents

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**ELECTROCHEMICAL STUDIES OF VITAMIN A AND
VITAMIN E IN ORGANIC SOLVENTS**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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**ELECTROCHEMICAL STUDIES OF VITAMIN A AND
VITAMIN E IN ORGANIC SOLVENTS**

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“Cultivate the habit of being grateful for every good thing that comes to you, and to give thanks continuously. And because all things have contributed to your advancement, you should include all things in your gratitude.”

— Ralph Waldo Emerson

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Abstract

Electrochemical studies were conducted on 6 major forms of vitamin A, namely; retinol, retinal, retinoic acid, retinyl acetate, retinyl palmitate and beta-carotene (β -Car) under similar experimental conditions using cyclic voltammetry (CV) and bulk controlled potential electrolysis (CPE) with coulometry. The compounds could all be reduced and oxidized in the aprotic organic solvents acetonitrile (CH_3CN) and dichloromethane (CH_2Cl_2), but the voltammetric responses varied depending on the solvent and different electrode surfaces (Pt and GC). Among the six different forms of vitamin A, retinal displays chemically reversible reductive behavior during CV experiments. Retinal can be voltammetrically reduced in CH_3CN in two one-electron processes to first form the anion radical ($\text{R}^{\bullet-}$) and then the dianion (R^{2-}). The anion radical undergoes a reversible dimerization reaction to form the dimer dianion (R_2^{2-}). All three anion species (anion radical, dianion and dimer dianion) undergo hydrogen-bonding interactions with water that is present at millimolar levels in the solvent.

Interactions between vitamin species were examined. α -Tocopherol (vitamin E) is known to undergo $2e^-$ oxidation while β -Car (pro-vitamin A) also undergoes a $2e^-$ oxidation process. Cyclic voltammetry experiments indicated that the oxidative peak potential for α -tocopherol (α -TOH) was approximately +0.40 V more positive than the oxidative peak potential of β -Car. A solution of $\alpha\text{-TO}^+/\text{H}^+$ (prepared by chemical oxidation of α -TOH with 2NO^+) was reacted with a solution containing an equal molar amount of β -Car. Voltammetric monitoring indicated that α -TOH was quantitatively regenerated and $\beta\text{-Car}^{2+}$ was formed in high yield in a homogeneous two-electron transfer.

The electrochemical oxidation of α -TOH was performed in CH_3CN in the presence of varying amounts of water to understand how the water affected the overall oxidation mechanism. α -TOH can be electrochemically oxidised in a $-2e^-/-\text{H}^+$ process to form a diamagnetic cation that is long-lived in dry organic solvents but in the presence of

water quickly reacts to form a hemiketal. The oxidation peak potential of α -TOH measured during cyclic voltammetry experiments was found to shift to less positive potentials as increasing amounts of water were added to the CH_3CN , which was interpreted based on hydrogen-bonding interactions between the phenolic hydrogen atom and water. The results obtained for α -TOH have wide implications on how the electrochemical behavior of other phenols in organic solvents are interpreted.

Chapter 1

Introduction

1.1 General Introduction on Vitamins

Vitamins are organic compounds which are mainly obtained from food sources because they cannot be sufficiently generated by metabolic reactions. Even though only small amounts are needed, they are a form of essential nutrients which are necessary for the proper growth and development of organisms.¹ Thanks to the studies conducted to identify and isolate these vital compounds, there are now a total of 13 vitamins known to exist and they have been generally classified into fat-soluble and water-soluble species. Each group of vitamin has a specific role to play in performing different tasks to sustain the development of the body.² Inadequacy of any of the vitamins can lead to deficiency diseases.^{3,4} In order to prevent the human organism from reaching such a condition, it is crucial that a well-balanced diet is followed. It is possible to acquire nearly all the vitamins needed by eating a wide range of foods. In some cases, supplements are taken to substitute some food sources or to improve the overall vitamin intake to an optimum level. This has created much controversy on whether consuming vitamin supplements has a positive effect on health or does it lead to unknown side effects.⁵ In order to fully understand the desirable amount of supplements required, it is imperative to study vitamin chemistry and how they are being absorbed and converted within the body. The reaction mechanism that each vitamin undergoes in its human metabolic pathway needs to be well understood.

1.1.1 Background of Vitamin Research

Research on nutrition began long ago but it was not until the early 20th century that the development of science had led to the realization of its huge significance in physiology.⁶⁻⁹ Previous to this time, studies were conducted in search of an association

between food and the diseases that were occurring. It was believed that natural food sources consisted of mainly proteins, calories, salts and water, even though there was evidence to suggest otherwise. Nutritional studies had focused much on the energy that can be acquired from various foods rather than taking into account the chemical composition that was supplied in the diet.¹⁰ Diseases that occurred were often associated with bacterial infection and toxins existing in the food supply rather than a deficiency symptom in nutrition. The idea of a disease occurring due to a lack of essential substances in food sources was not widely accepted until the beginning of the 20th century.¹¹ This spurred on much research on food nutrition, in relation to the illnesses which were later realized to be deficiency diseases, and brought about new insights on these vital compounds that exist in natural food sources.¹²⁻¹⁴ It is through the research and questions raised by many scientists that led to the discovery of these substances, which although present in small quantities are of paramount significance in the wellbeing of organisms.^{15,16} The term 'vitamine' was coined by Casimir Funk because the compound he isolated was thought to be vital to life and contained an amine group.^{17,18} It was later discovered that not all compounds which can be considered as a vitamin contain the amine group, hence the 'e' at the end of 'vitamine' was removed.¹⁹

1.1.2 The Importance of Vitamins

When studies showed the significance of vitamins and the positive impact they can have on the health of individuals, people started believing in the importance of these individual compounds. Vitamins are believed to be a prerequisite for the body in order to remain in "top condition".²⁰ Plans were also put in place to fortify food with the necessary vitamins. People in more affluent countries started taking supplements thinking that they were good for their overall health.⁵ Some studies had also shown possible antioxidant properties of these vitamins and proposed their use as anticancer agents.²¹ Nevertheless, in many situations the exact biochemical reactions and effects on bodily functions are still

not fully understood. These concerns have driven additional kinds of research on nutrition such as dietary intake regulations,²² and analytical methods developed to extract vitamins from food sources which still retains their bioactivity.²⁰ Epidemiological studies have been carried out to observe how cancer risks can be lowered by providing different nutritional controls and regulated for different groups of people.²³⁻²⁷ Future research focus needs to be on not only studying the natural form of the vitamins that are ingested, but to study the exact reaction pathway that the molecules undergo in order to understand the cause and effect of their action in vivo.

1.1.3 The use of Electrochemical Methods to Study Potential Biological Functions of Vitamins

Present electrochemical studies in the area of molecular electrochemistry of vitamins are very inadequate.²⁸ Most electrochemical studies on vitamins simply involve using a voltammetric technique to quantify the amount of a vitamin in a model test environment, which in real physiological samples seldom leads to satisfactory result due to the large number of potential interferences (other redox active molecules) that are present. Nevertheless, proper investigations using voltammetric techniques can be very useful as a powerful tool in conceptualizing the bioactivity role and chemical reactions that vitamins can undergo.²⁹ The electron transfer processes and the reaction mechanism of vitamins can be studied with the use of electrochemical methods. In this thesis, vitamins A and E are focused upon and a brief introduction follows on what electrochemical studies have been performed on these two vitamins up to the present date.

1.2 General Introduction of Vitamin A

Vitamin A comprises a group of fat-soluble compounds which are made up of retinoids and carotenoids (vitamin A precursor).³⁰ They cannot be generated within the body in adequate amounts and have to be acquired from dietary intake. There are

generally two different types of food origin. Food of animal origin provides preformed vitamin A which has been converted by the animal from the precursor molecules. These food sources include liver, milk and eggs. Food of plant origin are rich in carotenoids, which are also known as provitamin A and act as precursors to the active vitamin A. Rich sources of carotenoids typically comes from fruits and vegetables. There are many forms of carotenoids and not all of them can be chemically converted to vitamin A. One of the most active precursor forms is β -carotene.³¹ It has been shown that β -carotene undergoes central cleavage to form two molecules of vitamin A (retinal).³²⁻³⁵

Scheme 1.1: Metabolic pathway and absorption of β -carotene and retinyl esters from the intestine through enterocyte into the lymph and blood. [Adapted from Ref. (36)]

The metabolic pathway that vitamin A undergoes takes place in the intestine after ingestion and results in the compound eventually stored in the liver. Vitamin A sources in food are extracted during the digestion process and after crossing the intestinal lumen, undergo a series of chemical conversions in the enterocyte (Scheme 1.1). The retinyl esters formed in enterocytes are secreted into the bloodstream through the lymphatic system. Chylomicron circulates in the bloodstream and any remnants of vitamin A will be

absorbed by the liver. The retinyl esters which are transported together with the chylomicron will be stored within the liver until it is needed by the body.^{20,36}

1.2.1 Different Forms of Vitamin A

The general structure of vitamin A consist of a β -ionone ring, an unsaturated side chain with different terminal groups such as an alcohol, aldehyde, acid and ester.³⁷ These compounds together collectively comprise the major vitamers of vitamin A and are shown in Scheme 1.2. Vitamin A is also comprised of carotenoids, which are a form of provitamin A. β -carotene, which is studied in this thesis, is composed of two β -ionone rings and a long unsaturated chain in between. The structure of β -carotene was determined by Karrer in 1930s.^{38,39} It is understood that different parts of the molecules play a role in the correct functioning of the vitamin A activity. Changes to these specific parts of the structure, such as opening of the carbon ring and displacement of the double bond in a different position on the carbon chain, will render the function lost.^{39,40}

Scheme 1.2: Structures of vitamin A species existing in different forms.

1.2.2 Properties and Function of Vitamin A

Vitamin A is involved in many different functions in the body such as strengthening of the immune system, vision, reproduction, cellular communication and

differentiation, and it is also believed to have antioxidant activity.^{30,41-44} Studies have also shown its potential in reducing the risk of cancers.^{29,45} Deficiency in vitamin A will lead to various diseases and impede healthy growth and development.⁴⁶ Studies have shown that retinal is needed in order to maintain a healthy ocular system. Deficiency can lead to night blindness and various eye diseases.⁴³ A lack of vitamin A can increase the vulnerability to bacterial, viral or parasitic infections. Such observations provide evidence that vitamin A is vital in maintaining the defence mechanisms in the body. Regions in the world with a lack of proper nutrition tend to suffer from vitamin deficiency. Children who suffer from vitamin A deficiency have to be given supplements to ensure their survival.⁴⁷ Aid programmes that use supplements have lowered the mortality rate of children significantly in affected regions. Epidemiological studies have shown potential beneficial effects of vitamin A in lowering the incidence of cancers, possibly by it behaving as an antioxidant.⁴⁸⁻⁵¹ Despite evidence of a largely positive impact vitamin A has on bodily functions, there are also studies which present undesirable results.⁵²⁻⁵⁵ Such controversy has led to a need to really understand the primary function of the vitamin at a molecular level. A comprehensive understanding of the behavior of vitamin A can only be achieved if all of its fundamental reactions are known. Vitamins in food sources are often taken together with other components, and its reaction in the body becomes very complicated to study. There are many factors which could govern the metabolic pathways. Therefore, in order to know the actual role and effect of vitamin A, it is necessary to understand the type of reactions it may undergoes by itself, such as those that can be initiated by electrochemical oxidation or reduction.

1.2.3 Chemical studies of Vitamin A

Many studies have been performed to obtain the amount of vitamin A that exists in various food sources. These often involved the use of analytical methods such as spectroscopy and voltammetry.^{56,57} As food is often made up of a mixture of substances,

studies on the interaction between different types of important vitamins have been performed. Since vitamin A is one of the vitamins that has been proposed to have important antioxidant functions, possible synergistic interactions with other antioxidant functioning vitamins such as vitamin C and vitamin E has been proposed.⁵⁸⁻⁶⁰

1.3 General introduction of Vitamin E

Vitamin E is an essential compound for reproduction in many mammals which was discovered in 1922 by Evans and Bishop.⁶¹ Its role in reproduction was found when it was observed that pregnant rats fed with an isolated diet failed to reproduce until lettuce or whole wheat was given to them. The substance lacking in such a diet was later found to be present in wheat germ oil. The vitamin was isolated in 1936 in a crystalline form and was latter named "tocopherol" (TOH), which is derived from the Greek word "tokos" (child birth) and "pherein" (to bear). The suffix "-ol" indicates that it contains an alcohol group.^{62,63}

1.3.1 Different forms of Vitamin E

There are eight forms of vitamin E which are classified into two major groups; tocopherols and tocotrienols. Each group is further categorized into four prefixes, namely alpha (α), beta (β), gamma (γ) and delta (δ).³⁰ These compounds have all been isolated from plant sources. The structures of all the compounds are comprised of a chromanol ring with a hydrophobic side chain. There are four tocopherols (TOH) that have a fully saturated phytyl side chain. The other four tocotrienols have similar structures except that they have double bonds at position 3', 7' and 11' of the side chain (Scheme 1.3). The individual forms (α -, β -, γ - and δ -) of the tocopherols and tocotrienols differ by the number and the position of the methyl substitution in the chromanol ring. Besides the naturally occurring form of vitamin E, there are also synthetic forms that are available commercially. Naturally occurring α -TOH is assigned the name RRR- α -TOH to

differentiate it from the synthetic form. Synthetic α -TOH, named all-rac-TOH, consists of a mixture of eight isomers while only 12.5% of it is in the RRR- form.⁶⁴

Scheme 1.3: Structures of vitamin E.

Among the different forms of vitamin E, α -TOH is the most abundant and most biologically active.⁶⁵ It is the preferred form that is actively maintained within the body. Naturally occurring tocopherols in food when ingested are absorbed from the intestine into the enterocyte. Within the enterocyte, vitamin E then resides within chylomicrons while it is secreted into the bloodstream. On entering the bloodstream, the chylomicrons are attacked by lipoprotein lipase, and hydrolysis of the core triglycerides results in the formation of chylomicron remnants. The chylomicron remnants, containing the major fraction of absorbed vitamin E, are then absorbed by the liver. The liver then secretes the newly absorbed vitamin E into the plasma where it reside within very low density lipoproteins (VLDLs).⁶⁶ α -TOH (compared to the β , γ and δ -forms) is preferentially secreted and this discrimination explains why there is a higher amount of it in the blood.⁶⁷ The liver also prefers secreting RRR- α -TOH compared to the other stereoisomers and this differentiation has been attributed to a hepatic tocopherol-binding protein known as the α -

Tocopherol Transfer Protein (α -TTP) in the liver.⁶⁸ The improved retention of the natural form makes the bioavailability of RRR- α -TOH approximately twice that of synthetic vitamin E.⁶⁹

1.3.2 Properties and functions of Vitamin E

It is widely believed that vitamin E's major role in mammalian tissues relates to its ability to act as an antioxidant where living cell membranes are essentially prevented from turning rancid and decomposing.⁷⁰⁻⁷³ In the body, free radicals are formed during normal metabolism (lipid peroxidations) as well as through exposure to environmental factors such as smoke and pollutants (autoxidations). Living cell membranes become susceptible to oxidative damage by free radicals. The lipid-soluble vitamin E plays a role in intercepting the free radicals and hence prevents chain reactions of lipid peroxidation.⁷⁴ The process involving a free radical chain reaction begins with an initiation step where the carbon-centered radical ($R^\bullet$) is formed by the removal of a hydrogen atom from a lipid molecule (RH). This can either be derived from an enzyme-catalyzed electron transfer reaction or under a nonenzymatic process caused by heat or light. The $R^\bullet$ radical reacts rapidly with oxygen to form a peroxy radical ($ROO^\bullet$) which in turn reacts with another lipid molecule (RH) to form a lipid hydroperoxide ($ROOH$) and a new $R^\bullet$. This propagation cycle can be terminated by an antioxidant such as vitamin E. Vitamin E (TOH) acts as a chain breaking antioxidant by reacting with the peroxy radical ($ROO^\bullet$) to form a molecule of lipid hydroperoxide ($ROOH$) and the tocopheroxy radical ($TO^\bullet$). The tocopheroxy radical ($TO^\bullet$) continues to react with another molecule of peroxy radical ($ROO^\bullet$) which eventually terminates the chain reaction.⁷⁰

When TOH reacts with free radicals in the lipid peroxidation process, it loses its antioxidant activity. The activity has been proposed to be restored by other antioxidants such as vitamin A⁷⁵ and vitamin C regenerating the starting form.^{76,77}

While it is possible for vitamin E to act as a sacrificial compound to limit the damage of cell membranes, a number of new properties of vitamin E have been proposed in recent years, and this has led to a degree of controversy over whether vitamin E is just an antioxidant.^{21,74,78-83} Some of the new proposed functions include inhibiting the activity of protein kinase C (PKC) which is an important cell-signaling molecule,⁸⁴⁻⁸⁷ affecting the expression and activities of molecules and enzymes in immune and inflammatory cells, inhibiting platelet aggregation, and enhanced vasodilation.^{68,88} Inhibition of protein kinase C (PKC) was found to affect smooth muscle cell proliferation which was induced by α -TOH.^{80,81} This function is not believed to be related to its antioxidant action. β -TOH was found to be ineffective at the inhibition of PKC but prevented the inhibitory effect of α -TOH. γ -TOH and δ -TOH had no effect on PKC.^{80,81} The mechanism of α -TOH inhibiting PKC may be due to the decrease in the generation of diacylglycerol which is a lipid that activates PKC activity.⁸⁹ This action does not show a direct link to α -TOH antioxidant activity. Addition of α -TOH to PKC does not result in the inhibition and this suggests there is no direct interaction between α -TOH and PKC during the inhibition action.⁸¹ There might be important mechanistic features associated with α -TOH that is the reason for its preferential retention in the lipid membranes and that causes it to interact with PKC in a special way. This characteristic is probably not shared with the β -, γ -, δ -forms as they have different activity from α -TOH.⁹⁰

1.3.3 Electrochemical Studies of Vitamin E

Unlike vitamin A (except for β -carotene) there have been many detailed voltammetric studies performed on vitamin E. The basis of the reactions involved in antioxidant functions of vitamin E can be described as electron transfer reactions and/or

hydrogen atom transfer processes and as such it should exhibit electroactivity.⁹¹ Among the various forms of vitamin E, α -TOH displays some unique electrochemical properties which may be related to its proposed non-antioxidant capacity. In order to establish chemical reasons for the unique non-antioxidant biological functions and behavior of α -TOH, it is necessary to recognize all the different oxidized forms of α -TOH. Electrochemical methods have been used to evaluate the properties of vitamin E as the in vivo behavior may not simply be represented by eqs 1.3 and 1.4.⁹²

Electrochemical studies of vitamin E have shown that α -, β -, γ - and δ - forms undergoes a series of chemically reversible proton and electron transfers to form oxidized compounds of different lifetimes in aprotic organic solvents such as acetonitrile (CH_3CN) and dichloromethane (CH_2Cl_2).⁹² The electrochemical mechanism for the oxidation of vitamin E on solid electrodes is given in Scheme 1.4.⁹³⁻⁹⁸ The reaction pathway can proceed differently depending on the presence or absence of organic soluble acids and bases during the oxidation. Pathway 1 shows the reaction occurring in acid conditions, pathway 2 shows how it proceeds in the absence of acid or base and pathway 3 is performed under basic conditions (Scheme 1.4). Most of the studies had been performed using α -TOH, but the same sequence applies to the β , γ and δ -tocopherols (and tocotrienyls) but with their fully oxidized forms having substantially different lifetimes.

Scheme 1.4: Electrochemical induced transformation of tocopherol in CH_3CN or CH_2Cl_2 . One resonance structure is displayed for each compound. The listed potentials are values versus Fc/Fc^+ . The counterions for the charged species are supporting electrolyte cation $[\text{Bu}_4\text{N}^+]$ and anion $[\text{PF}_6^-]$ and the " H^+ " released during the oxidation likely exists coordinated to trace water or the solvent. [Adapted from Ref. (92)]

In the absence of acid or base, α -TOH is oxidized at approximately $+0.5 (\pm 0.1)$ V vs. Fc/Fc^+ by one electron to form the radical cation (α -TOH $^{\bullet+}$). The radical cation (α -TOH $^{\bullet+}$) quickly deprotonates to form the neutral radical (α -TO $^\bullet$). The oxidation potential of α -TO $^\bullet$ is approximately $+0.1 (\pm 0.1)$ V vs. Fc/Fc^+ , and is less than that of α -TOH, hence α -TO $^\bullet$ is immediately further oxidized at the electrode surface (or homogeneously) to form the phenoxonium cation (α -TO $^+$).^{97,100} The oxidation process occurs via an ECE

mechanism, where E represents an electron transfer step and C represents a chemical step.⁹⁴ There is another possibility where the second electron-transfer step occurs via a homogeneous disproportionation mechanism which would still generate a phenoxonium cation ($\alpha\text{-TO}^+$) (Scheme 1.5).^{97,101} Regardless of the reaction pathway, oxidation of $\alpha\text{-TOH}$ is fully chemically reversible on a fast CV (milliseconds) and electrolysis (hours) timescale, provided the water content of the CH_3CN is low. This allows the starting material ($\alpha\text{-TOH}$) to be regenerated quantitatively from $\alpha\text{-TO}^+$ when a reducing potential is applied.^{94,95} Besides inducing the oxidative transformation electrochemically, the conversion from $\alpha\text{-TOH}$ to $\alpha\text{-TO}^+$ can be achieved using an oxidizing agent such as NO^+ via a homogeneous chemical reaction.⁹⁵

Scheme 1.5: Possible homogeneous electron-transfer reaction during oxidation of $\alpha\text{-TOH}$. [Adapted from Ref. (97)]

Electrochemical studies have also shown that the phytyl chain of the tocopherol molecule does not affect its electrochemical properties. The same voltammetric behavior is observed when the phytyl chain is substituted with a methyl group in the model compound.^{95,96,100}

A cyclic voltammogram of the oxidation of $\alpha\text{-TOH}$ forming $\alpha\text{-TO}^+$ followed by the reverse reduction from $\alpha\text{-TO}^+$ back to $\alpha\text{-TOH}$ is shown in Figure 1.1. The position of the forward and reverse peaks are affected by the trace moisture content of the solvent.¹⁰² The forward and reverse processes involve two one-electron transfers and one proton transfer. Each of the electron transfer step takes place at different potentials and they are interspersed by a proton transfer step.

Figure 1.1: Cyclic voltammogram of 1×10^{-3} M α -tocopherol in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K recorded at 1mm diameter Pt disk electrode at a scan rate of 0.1 V s^{-1} . [Adapted from Ref. (99)]

A recent study on the voltammetric properties of all the tocopherols performed under the same conditions indicated that β -TOH, γ -TOH and δ -TOH undergo the same mechanism as α -TOH (Scheme 1.4). The significant difference between the different forms is related to the lifetime of the phenoxonium cation (TO^+). $\alpha\text{-TO}^+$ survives in dry CH_3CN for at least several hours, $\beta\text{-TO}^+$ is stable for a number of minutes and $\gamma\text{-TO}^+$ and $\delta\text{-TO}^+$ are stable for less than a second.¹⁰³ This large difference in the lifetimes of the phenoxonium cations among the different forms of the tocopherols is noteworthy, and also correlates with their biological activities.

1.4 Aim of the project

In order to appreciate the action that each individual vitamin might play biologically, it is necessary to understand the possible chemical reactions that they can undergo under relatively mild oxidizing or reducing conditions that can occur in natural systems. To date, the voltammetric behavior of vitamin A has usually been presented based on the individual species (vitamers), rather than comparing the results of all the various forms of vitamin A relative to each other. To understand how each of the species might be interrelated to one another, it is necessary to investigate and compare the different voltammetric behavior of each of the vitamins.

In this thesis, electrochemical studies were conducted on the major forms of vitamin A and the fully methylated form of vitamin E (α -TOH). The voltammetric

behavior of vitamin A and E were examined in different solvent systems and at glassy carbon and platinum electrodes. Noting that both vitamin A and E are fat soluble vitamins, the reaction conditions used were the organic solvents acetonitrile and dichloromethane. Different scan rates, concentrations and temperatures were used to study the reaction pathway of vitamins A and E. The number of electrons transferred for each voltammetric response were calculated using controlled potential electrolysis with coulometry. From the results obtained, the reaction mechanisms of the reduced or oxidized species were modeled using digital simulation techniques which enabled kinetic and thermodynamic parameters associated with the homogeneous and heterogeneous reactions to be determined for several of the vitamins.

The interaction between different types of vitamins that co-exist in nature was also studied. α -Tocopherol, is understood to undergo an electrochemical $2e^-$ oxidation, which is the same as known to occur for β -carotene (a provitamin A). Therefore, experiments were performed in order to assess whether the oxidized form of vitamin E was able to interact with β -carotene and thereby be regenerated.

1.5 References

1. Piro, A.; Tagarelli, G.; Lagonia, P.; Tagarelli, A.; Quattrone, A. *Ann. Nutr. Metab.* **2010**, *57*, 85-88.
2. Carpenter, K. J. *J. Nutr.* **2003**, *133*, 3023-3032.
3. Carpenter, K. J. *J. Nutr.* **2003**, *133*, 638-645.
4. Carpenter, K. J. *J. Nutr.* **2003**, *133*, 975-984.
5. Carpenter, K. J. *J. Nutr.* **2003**, *133*, 3331-3342.
6. Rosenfeld, L. *Clin. Chem.* **1997**, *43*, 680-685.
7. Sharman, I. M. *Endeavour.* **1977**, *1*, 97-102.
8. Sydenstricker, V. P. *Proc. Nutr. Soc.* **1953**, *12*, 256-269.
9. Chick, H. *Progr. Food Nutr. Sci.* **1975**, *1*, 1-20.
10. "Sir Frederick Hopkins - Nobel Lecture: The Earlier History of Vitamin Research".
Nobelprize.org.
http://www.nobelprize.org/nobel_prizes/medicine/laureates/1929/hopkins-lecture.html (accessed 12th August, 2012).
11. "Christiaan Eijkman - Nobel Lecture: Antineuritic Vitamin and Beriberi".
Nobelprize.org.
http://openx.nobelprize.org/nobel_prizes/medicine/laureates/1929/eijkman-lecture.html (accessed 16th June, 2013).
12. McCollum, E. V.; Davis, M. *J. Biol. Chem.* **1915**, *23*, 181-230.
13. McCollum, E. V.; Davis, M. *J. Biol. Chem.* **1914**, *19*, 245-250.
14. McCollum, E. V.; Simmonds, N.; Pitz, W. *J. Biol. Chem.* **1916**, *27*, 33-43.
15. "The Nobel Prize and the Discovery of Vitamins". Nobelprize.org.
http://www.nobelprize.org/nobel_prizes/themes/medicine/carpenter/ (accessed 20th June, 2013).
16. Hopkins, F. G. *J. Physiol.* **1912**, *44*, 425-460.

17. Funk, C. J. *State Med.* **1912**, 20, 341-368.
18. Griminger, P. J. *Nutr.* **1972**, 102, 1105-1113.
19. Drummond, J. C. *Biochem. J.* **1920**, 14, 660.
20. Ball, G. F. M. *Vitamins: their role in the human body*, 1st Ed.; Blackwell Pub.: Oxford, U.K.; Blackwell Professional Pub.: Ames, Iowa, 2004.
21. Melton, L. *New Scientist* **2006**, 191, 40-43.
22. Hathcock, J. N. *Vitamin and Mineral Safety*, 2nd Ed.; CRN, 2004.
23. Reddy, B. S. *Can. Med. Assoc. J.* **1980**, 123, 850-856.
24. Wynder, E. L. *Cancer Res.* **1975**, 35, 3388-3394.
25. Burkitt, D. P. *Dis. Colon Rectum.* **1993**, 36, 1071-1082.
26. Trowell, H. *Am. J. Clin. Nutr.* **1972**, 25, 926-932.
27. Kay, R. M.; Truswell, A. S. *Am. J. Clin. Nutr.* **1977**, 30, 171-175.
28. De Abreu, F. C.; Ferraz, P. A. L.; Goulart, M. O. F. *J Braz. Chem. Soc.* **2002**, 13, 19-35.
29. Litwack, G. *Vitamin A: vitamins and hormones, volume 75*, 1st Ed.; Elsevier Academic Press: Burlington, 2007.
30. Sardesai, V. M. *Introduction to clinical nutrition*, 2nd Ed.; Marcel Dekker: New York, 2003.
31. Olson, J. A. In *Vitamin A. Present knowledge in nutrition*, 7th Ed.; Ziegler, E. E.; Filer, L. J. Jr., Eds.; Washington, DC: ILSI Press, 1996, 109-119.
32. Olson, J. A. *J. Nutr.* **1989**, 119, 105-108.
33. Wang, X. D.; Krinsky, N. I. In *Subcellular Biochemistry, Volume 30: Fat-Soluble Vitamins- The Bioconversion of β -Carotene into Retinoids*, Quinn, P. J.; Kagan, V. E. Eds.; Plenum Press: New York, 1998, chapter 7.
34. Moore, T. *Biochem. J.* **1930**, 24, 692-702.
35. von Euler, B.; von Euler, H.; Hellström, H. *Biochem. Z.* **1928**, 203, 370-384.

36. Erdman Jr, J. W.; Bierer, T. L.; Gugger, E. T. *Ann. N. Y. Acad. Sci.* **1993**, *691*, 76-85.
37. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)
Nomenclature of Retinoids. *Eur. J. Biochem.* **1982**, *129*, 1-5.
38. Karrer, P.; Helfenstein, A.; Wehrle, H.; Wettstein, A. *Helv. chim. Acta.* **1930**, *13*, 1084-1099.
39. Karrer, P. *Chem. Rev.* **1934**, *14*, 17-30.
40. "Paul Karrer - Nobel Lecture: Carotenoids, Flavins and Vitamin A and B2".
Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1937/karrer-lecture.html (accessed 19th June, 2013).
41. Wolf, G. *FASEB J.* **1996**, *10*, 1102-1107.
42. Krinsky, N. I. *Ann. N. Y. Acad. Sci.* **1998**, *854*, 443-447.
43. Wald, G. *Science* **1968**, *162*, 230-239.
44. Dawson, M. I. *Curr. Pharm. Des.* **2000**, *6*, 311-325.
45. Nagao, A. *J. Nutr.* **2004**, *134*, 237S-240S.
46. Feskanich, D.; Singh, V.; Willett, W. C.; Colditz, G. A. *JAMA.* **2002**, *287*, 47-54.
47. UNICEF. Vitamin A Supplementation: A Decade of Progress, UNICEF: New York, 2007.
48. Palozza, P.; Krinsky, N. I. *Methods Enzymol.* **1992**, *213*, 403-420.
49. Krinsky, N. I. *Nutrition*, **2001**, *17*, 815-817.
50. Ziegler, R. G. *J. Nutr.* **1989**, *119*, 116-122.
51. Peto, R.; Doll, R.; Buckley, J. D.; Sporn, M. B. *Nature* **1981**, *290*, 201-208.
52. Krinsky, N. I.; Johnson, E. J. *JMAM.* **2005**, *26*, 459-516.
53. Denke, M. A. *JAMA*, **2002**, *287*, 102-104.
54. Caire-Juvera, G.; Ritenbaugh, C.; Wactawski-Wende, J.; Snetselaar, L. G.; Chen, Z. *Am. J. Clin. Nutr.* **2009**, *89*, 323-330.

55. ATBC-Study Group. *N. Engl. J. Med.* **1994**, 330, 1029–1035.
56. Valentin Pfund, B.; Bond, A. M.; Hughes, T. C. *Analyst* **1992**, 117, 857-861.
57. Ziyatdinova, G.; Ziganshina, E.; Budnikov, H. *Talanta* **2012**, 99, 1024-1029.
58. Truscott, T. G. In *Functions of Vitamins Beyond Recommended Dietary Allowances*, vol. 55. *Synergistic effects of antioxidant vitamins*, Walter, P.; Hornig, D.; Moser, U., Eds.; Karger: Basel, 2001, 68–79.
59. Palozza, P.; Krinsky, N. I. *Arch. Biochem. Biophys.* **1992**, 297, 184-187.
60. Yeum, K. -J.; Dos Anjos Ferreira, A. L.; Smith, D.; Krinsky, N. I.; Russell, R. M. *Free Rad. Biol. Med.* **2000**, 29, 105-114.
61. Evans, H. M.; Bishop, K. S. *Science* **1922**, 56, 650-651.
62. Evans, H. M.; Emerson, O. H.; Emerson, G. A. *J. biol. Chem.* **1936**, 113, 313.
63. Evans, H. M. *Vit. Horm.* **1962**, 20, 379-387.
64. Burton, G. W.; Traber, M. G.; Acuff, R. V.; Walters, D. N.; Kayden, H.; Hughes, L.; Ingold, K. U. *Am. J. Clin. Nutr.* **1998**, 67, 669-684.
65. Sheppard, A. J.; Pennington, J. A. T.; Weihrauch, J. L. In *Vitamin E in health and disease*, Packer, L.; Fuchs, J. eds.; Marcel Dekker, Inc.: New York, 1993, 9-31.
66. Kayden, H. J.; Traber, M. G. *J. Lipid Res.* **1993**, 34, 343-358.
67. Traber, M. G. *Biofactors.* **1999**, 10, 115-120.
68. Herrera, E.; Barbas, C. *J. Physiol. Biochem.* **2001**, 57, 43-56.
69. Horwitt, M. K. *Am. J. Clin. Nutr.* **1980**, 33, 1856-1860.
70. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, 19, 194-201.
71. Niki, E.; Noguchi, N. *Acc. Chem. Res.* **2004**, 37, 45-51.
72. Wang, X.; Quinn, P. J. *Mol. Membr. Biol.* **2000**, 17, 143-156.
73. Finkel, T.; Holbrook, N. J. *Nature* **2000**, 408, 239-247.
74. Traber, M. G.; Atkinson, J. *Free Rad. Biol. Med.* **2007**, 43, 4–15.

75. Bohm, F.; Edge, R.; Land, E. J.; McGarrey, D. J.; Truscott, T. G. *J. Am. Chem. Soc.* **1997**, *119*, 621-622.
76. Bruno, R. S.; Leonard, S. W.; Atkinson, J.; Montine, T. J.; Ramakrishnan, R.; Bray, T. M.; Traber, M. G. *Free Rad. Biol. Med.* **2006**, *40*, 689-697.
77. Bowry, V. W.; Stocker, R. *J. Am. Chem. Soc.* **1993**, *115*, 6029-6044.
78. Brigelius-Flohé, R.; Davies, K. J. A. *Free Rad. Biol. Med.* **2007**, *43*, 2-3.
79. Azzi, A. *Free Rad. Biol. Med.* **2007**, *43*, 16-21.
80. Ricciarelli, R.; Zingg, J. -M.; Azzi, A. *Biol. Chem.* **2002**, *383*, 457-465.
81. Azzi, A.; Stocker, A. *Prog. Lipid Res.* **2000**, *39*, 231-255.
82. Zingg, J. -M.; Azzi, A. *Curr. Med. Chem.* **2004**, *11*, 1113-1133.
83. Azzi, A.; Breyer, I.; Feher, M.; Pastori, M.; Ricciarelli, R.; Spycher, S.; Staffieri, M.; Stocker, A.; Zimmer, S.; Zingg, J. -M. *J. Nutr.* **2000**, *130*, 1649-1652.
84. Boscoboinik, D.; Szewczyk, A.; Hensey, C.; Azzi, A. *J. Biol. Chem.* **1991**, *266*, 6188-6194.
85. Tasinato, A.; Boscoboinik, D.; Bartoli, G. M.; Maroni, P.; Azzi, A. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12190-12194.
86. Özer, N. K.; Sirikci, Ö.; Taha, S.; San, T.; Moser, U.; Azzi, A. *Free Rad. Biol. Med.* **1998**, *24*, 226-233.
87. Azzi, A.; Boscoboinik, D.; Chatelain, E. Özer, N. K.; Stäuble, B. *Mol. Aspects Med.* **1993**, *14*, 265-271.
88. Traber, M. G. *J. Nutr.* **2001**, *131*, 3955-3975.
89. Kunisaki, M.; Bursell, S. E.; Umeda, F.; Nawata, H.; King, G. L. *Diabetes* **1994**, *43*, 1372-1377.
90. Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472-6477.
91. Blasco, A. J.; Crevillén, A. G.; González, M. C.; Escarpa, A. *Electroanalysis* **2007**, *19*, 2275-2286.

92. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
93. Webster, R. D. *Electrochem. Commun.* **1999**, 1, 581-584.
94. Williams, L. L.; Webster, R. D. *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.
95. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D. *J. Org. Chem.* **2005**, 70, 10466–10473.
96. Lee, S. B.; Willis, A. C.; Webster, R. D. *J. Am. Chem. Soc.* **2006**, 128, 9332-9333.
97. Yao, W. W.; Peng, H. M.; Webster, R. D.; Gill, P. M. W. *J. Phys. Chem. B* **2008**, 112, 6847-6855.
98. Peng, H. M.; Webster, R. D. *J. Org. Chem.* **2008**, 73, 2169-2175.
99. Webster, R. D. *Chem. Rec.* **2012**, 12, 188-200.
100. Svanholm, U.; Bechgaard, K.; Parker, V. D. *J. Am. Chem. Soc.* **1974**, 96, 2409-2413.
101. Costentin, C. *Chem. Rev.* **2008**, 108, 2145-2179.
102. Tan, Y. S.; Chen, S. S.; Hong, W. M.; Kan, J. M.; Kwek, E. S. H.; Lim, S. Y.; Lim, Z. H.; Tessensohn, M. E.; Zhang, Y.; Webster, R. D. *Phys. Chem. Chem. Phys.* **2011**, 13, 12745-12754.
103. Wilson, G. J.; Lin, C. Y.; Webster, R. D. *J. Phys. Chem. B* **2006**, 110, 11540-11548.

Chapter 2

Experimental Section

2.1 Introduction to the Electrochemical Procedures used in the Research

The experiments performed in this thesis used various standard electrochemical methods such as cyclic voltammetry (CV), square wave voltammetry (SWV), rotating disc electrode voltammetry (RDEV) and bulk controlled potential electrolysis with coulometry (chronoamperometry under electrolysis conditions). Different methods were used to study electrode processes that take place for different vitamers depending on the nature of study. When the experiments involved kinetic studies and the rate constants of the chemical steps were to be determined, digital simulation techniques were used. In the case where the experiments required accurate measurement of water content for various solutions, the Karl Fischer coulometric titrator was used.

2.2 Electrochemical Procedures

2.2.1 Introduction to Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) is a widely used electroanalytical technique mainly utilized for determining formal electrode potentials of compounds and for studying homogeneous mechanisms following heterogeneous electron transfer. It has become a very popular technique for initial electrochemical studies of new systems. Its wide applicability in the study of oxidation and reduction (Redox) reactions makes it a useful tool for the investigation on the electrochemistry of electroactive substances.^{1,2}

On the potential versus time scale (Figure 2.1*a*), the potential is held at the starting potential for a few seconds to equilibrate the system. For a reduction process, the potential is scanned in the negative potential direction until it reaches the switching potential, where the scan direction is reversed towards more positive potentials and the current is measured simultaneously. To decide which direction (positive or negative)

should be initially scanned will depend on whether the sample used for testing is to undergo an oxidation or reduction process (which is often not known for new samples).

Figure 2.1: (a) A triangular wave form measure potential (E) as a function of time (t). (b) Cyclic Voltammogram of current (i) against potential (E) for the reduction of A in the forward scan followed by oxidation of $A^{\bullet-}$ in the reverse scan. [Adapted from Ref. (2)]

A typical cyclic voltammogram has axis of current plotted against potential as shown in Figure 2.1*b*. For a reduction reaction, the potential starts from an initial potential and is scanned towards more negative potentials, where the concentration of the analyte (A) at electrode surface starts to decrease when the potential at the electrode approaches the formal potential of A . More A will be drawn to the electrode surface due to the increase in the concentration gradient. As the potential approaches the formal potential of A the current continues to increase. The scan will get to a point when the flux of A reaches its limit and stops increasing, while further scanning in the negative potential direction results in a drop in the current as more of A needs to diffuse to the electrode surface. At a sufficiently negative potential all of A is fully converted to $A^{\bullet-}$ at the electrode surface and the resulting current is limited by how fast A can reach the electrode surface (the "diffusion limited current" is obtained). The scan direction is reversed at a switching potential where the high concentration of $A^{\bullet-}$ at the electrode surface will start to decrease and be converted back to the starting material, A . The wave form is very similar for the reverse scan when compared to the forward scan. This completes one cycle of the scan.

When the anodic and cathodic peak current are of similar size, they are deemed to be a chemically reversible process where $(i_p^{\text{red}}/i_p^{\text{ox}}) = 1$ (i_p^{red} is the reductive peak current and i_p^{ox} is the oxidative peak current). However when $(i_p^{\text{red}}/i_p^{\text{ox}})$ is more than or less than 1, it may not be a chemically reversible reaction and in some circumstances it is possible to determine other information such as the kinetic values for follow up homogeneous chemical reactions.

There are different types of terminology used to describe the size of the forward and reverse peaks detected during CV as well as the separation between the forward and reverse peaks. In this work, the expressions *electrochemically reversible*, *electrochemically irreversible* and *electrochemically quasi-reversible* when used to describe CV experiments relate to the rates of heterogeneous electron transfer. Processes that have very fast electron transfer are termed reversible (or electrochemically reversible), while very slow electron transfer are irreversible, and quasi-reversible is between the two extremes. The effects of electrochemical reversibility are usually observable in CV experiments from the potential separation between the reductive (E_p^{red}) and oxidative (E_p^{ox}) peaks (ΔE_{pp}) for one process. Electrochemically slow or quasi-reversible processes have a large ΔE_{pp} . In contrast, the expressions *chemically reversible* or *chemically irreversible* when used to describe cyclic voltammograms relate to the life-times of the reduced or oxidized species in solution, and are observable from CV experiments based on the $i_p^{\text{red}}/i_p^{\text{ox}}$ ratios recorded at different scan rates. By varying the voltammetric scan rates, it is possible to obtain information on the fates of the reduced and oxidized compounds and whether they undergo follow-up homogeneous chemical reactions. For example, an "EC" mechanism is where there is an initial electron transfer ("E") followed by a chemical step ("C").³⁻⁵ There are numerous other possibilities (ECE, ECEC, EEC, etc) and cyclic voltammetry is particularly useful in studying these reactions. Digital

modelling of the CV data allows estimations of the rate and equilibrium constants for the heterogeneous electron transfer and homogeneous chemical reactions.

2.2.2 Introduction to Square-Wave Voltammetry (SWV)

SWV was developed by Osteryoung et al,⁶⁻⁸ and the potential-time waveform is shown in Figure 2.2 which been described extensively.⁹⁻¹¹ The current readings are taken at two points during each cycle. The first value is recorded at the end of the forward scan which happens during the first pulse while the second value is taken at the end of the reverse scan which occurs at the second pulse. The difference in current is then computed and generated graphically as a current versus potential waveform. The method is most appropriate for an electrochemically reversible system.

Figure 2.2: Pulse waveform of square wave voltammetry (bold line), showing the applied potential on the working electrode, is superimposed with staircase waveform which shows the ΔE_s after every cycle of the pulse (normal line). In every cycle, a current reading is taken at the point labeled forward sample for the forward scan while the point labeled reverse sample is taken for the reverse scan. [Adapted from Ref. (9)]

As the square-wave current is obtained from the difference between I_{forward} and I_{reverse} , it is possible to minimize the background reading as the peak current is offset from the difference of the two values. With lesser interference from the background response, SWV is most commonly used in analytical experiments since it allows voltammetric processes to be more easily observed, especially at low concentrations.

2.2.3 Introduction to Rotating Disc Electrode Voltammetry (RDEV)

Rotating disc electrode voltammetry (RDEV) is a voltammetric method which involves convection as the main form of mass transport instead of solely diffusion, which

occurs for CV and SWV experiments (Section 2.2.1). Diffusion transport plays a lesser role in RDEV experiments as convection is a more efficient mode of mass transport to the electrode surface.¹² The rotating disc electrode is typically constructed with a disc fixed within one end of a cylindrical Teflon material. The angular frequency is kept at around 2000 rpm (~ 33 Hz) which is within the rotation rate necessary to keep the flow laminar in order to generate reproducible results.¹³

Linear sweep voltammetry is used (CV in one direction) for the potential scanning of the rotating disc electrode to achieve steady state conditions.^{14,15} The use of rotating disc electrode allows solution to be constantly drawn in to the electrode surface from the bulk solution. The electroactive species will flow at an unchanging flow rate to the electrode surface and electrolyzed species which pass across the surface will go back to the bulk solution again. Fresh analyte is continuously fed to the electrode surface via the spinning action as well as convection and diffusion processes.^{16,17} The position of zero current can be determined with the use of RDEV as well as the diffusion coefficient (or number of electrons transferred) of the analyte molecules.

Rotating disk electrode voltammetry (RDEV) experiments were conducted with a Metrohm Autolab RDE-2 using a 3 mm diameter planar Pt electrode.

2.2.4 Introduction to Controlled Potential Bulk Electrolysis and Coulometry

In a coulometry experiment, the current is measured as a function of time at a fixed potential. It is used as a method to accurately calculate the number of electrons transferred in an electrochemical reaction during controlled potential electrolysis on the compound of interest. CV is first used to determine the appropriate potential for the electrolysis reaction. A constant potential of approximately 0.1 V more positive (for oxidation) or more negative (for reduction) than the voltammetric peak potential is held at the working electrode, allowing the compound to be exhaustively electrolyzed. Very large electrodes, typically mesh or plates, are used to ensure that all of the analyte can be

electrolyzed within a reasonable time frame and the solution is stirred to increase the mass transfer to the electrode surface. The homogeneous and heterogeneous electrode processes during the electrolysis have been describe by Monk and Fisher.^{18,19}

Depending on the electrode process, a reductive potential will typically generate negative current while an oxidative potential will give a positive current as a function of time. Integrating the current (the area under the current versus time graph) over the length of time of the electrolysis will give the total charge transferred during the process.^{20,21} When a known concentration of compound is used, the number of electrons transferred per molecule can be determined (eq 2.1).²²

$$Q=nNF \quad (\text{eq 2.1})$$

where

Q = charge, C

n = number of electrons,

F = Faraday's constant, 96485 C mol⁻¹

N = Number of moles converted, mol

Controlled potential coulometry experiments were conducted with a computer-controlled Metrohm Autolab PGSTAT302N potentiostat. The electrolysis was setup in a two compartment electrolysis cell divided by a porous membrane. One compartment was filled with the working electrode (platinum (Pt) wire mesh) for the electrolysis as well as a 1 mm diameter Pt or GC disk working electrode for the cyclic voltammetry experiment for setting the correct potential. An Ag wire miniature reference electrode (Cypress Systems) connected to the test solution via a salt bridge containing 0.5 M Bu₄NPF₆ in CH₃CN was used. Another Pt wire mesh was placed in the other compartment of the cell as the auxiliary electrode (AE) (Figure 2.3). The AE is placed in a separate compartment to prevent contamination of the analyte solution with products that are formed at the AE. This is a necessary precaution for conducting bulk electrolysis over a long period of time.

CHAPTER 2

Figure 2.3: Electrochemical cell use for the controlled potential electrolysis experiments.

Throughout the electrolysis, Ar gas was bubbled through the solutions to aid in increasing the mass transfer to the electrode surface as well as to remove dissolved oxygen gas which is itself reducible and will interfere with the reduction reaction.

Low temperature experiments were performed using a Julabo FP89-HL ultralow refrigerated circulator attached to the jacketed compartmentalized electrochemical cell. Circulating cooling solvent was allowed to flow into and out of the cell through the solvent inlet and outlet tube.

2.2.5 Electrochemical Cell Apparatus

The electrochemical cell setup consisted of three electrodes (Section 2.2.6) and a bubbler tube for the purging of the solution (Figure 2.4). The reference electrode is typically placed in close proximity to the working electrode so as to minimize unwanted "IR drop". This is associated with the uncompensated solution resistance which occurs between the working electrode and auxiliary electrode. Resistance and migration effects are also minimized with the use of a large concentration of supporting electrolyte.²³ The auxiliary electrode used typically has a larger surface area than the working electrode so as to prevent restraining the current flowing through the whole circuit.

All openings on the cell were stoppered in order to maintain an oxygen free environment during electrochemical testing. Ferrocene (Fc) was added to the solution near the end of the experiments to provide an internal standard with which to reference the potentials.^{24,25} The electrochemical cell was placed in a Faraday cage to minimize the electrical noise interference from the surroundings.

The three electrodes were connected to the potentiostat as shown in (Figure 2.5).

Figure 2.4: Setup of an electrolytic cell used for cyclic voltammetric experiments under normal conditions and low temperature conditions.

During the low temperature experiments, the cooling solvent was introduced into the jacketed electrochemical cell via a chilled solvent circulating bath (Section 2.6).

2.2.6 Electrodes

The working electrodes (WE) included 1 mm and 3 mm diameter platinum (Pt) disks and 1 mm diameter glassy carbon (GC) disk (Metrohm and Cypress Systems). Prior to each scan, the working electrodes were first polished on a polishing pad using aluminum oxide powder (grain size 0.3 μm). This is to ensure the reproducibility of the electrochemical scan by maintaining the cleanliness of the electrode surface.²⁶ The purpose of the working electrode is to monitor the response of the compound of interest (vitamins A and E). In conjunction with the working electrode, a platinum (Pt) wire auxiliary electrode (Metrohm) and an Ag wire miniature reference electrode (Cypress Systems) connected to the test solution via a salt bridge containing 0.5 M Bu_4NPF_6 (Section 2.9.2) in CH_3CN were used. The reference electrode is used to measure the potential applied to the working electrode when connected to the potentiostat, and to reduce the effects of uncompensated solution resistance. In Chapters 4 and 6, where experiments required the creation of very dry conditions, the internal filling solution of the reference electrode was prepared immediately prior to use to reduce the amount of contamination from water.

2.2.7 Instruments and Program Used

Cyclic voltammetry (CV) experiments were conducted with a computer-controlled Metrohm Autolab PGSTAT302N potentiostat and all voltammetric scans were carried out using the GPES program.

Figure 2.5: Connection of working, reference and auxiliary electrode to the potentiostat.

2.3 Karl-Fischer Titrator

The Karl-Fischer Titration is an analytical method use to measure the amount of water in the test solution via coulometric or voltammetric titration. The technique was first discovered by Karl Fischer in 1935.²⁷

The KF reaction was originally based on the use of pyridine where it acts as the base to form a complex with I_2 and SO_2 .^{28,29} Pyridine is harmful and has a toxic odour which makes it unpleasant to work with. Studies conducted by Barendrecht and Verhoff showed that pyridine could be replaced with another base without affecting the reaction mechanism.³⁰⁻³⁴ Based on this understanding, Scholz developed a pyridine-free reagent by using imidazole as the base.^{35,36}

2.3.1 Theory of Karl-Fischer Titrator

The Karl Fischer coulometric titration is conducted based on the standard reaction as shown in the following equations.

The I_2 present is first generated electrochemically by anodic oxidation in the anolyte solution (eq 2.4). This occurs at the generator electrode (Figure 2.6) where the

anolyte is separated from the catholyte by a diaphragm, and hydrogen gas is generated by the cathodic reduction.

Figure 2.6: An illustration of the setup of a Karl-Fischer Titrator and the electrochemical reaction that is occurring at the generator electrode and measuring electrode. [Adapted from Ref. (37)]

The anolyte consist of iodide, sulfur dioxide, imidazole and solvent (methanol) where iodide converts to iodine via a $2e^-$ process. One molecule of I_2 will react with one molecule of H_2O (eq 2.3). Therefore, the charge needed for 1 mol of H_2O will be $2 \times$

96485 coulombs. The amount of water reacted can be directly associated with the amount of iodine generated and hence calculated by recording the current and time of the reaction.

The end of the titration process is marked by a drop in the voltage measured at two Pt wire indicator electrodes. A constant current of $-2\ \mu\text{A}$ is applied to the indicator electrodes. Once free iodine is generated in the anolyte compartment, the potential needed to maintain the $-2\ \mu\text{A}$ drops since the iodine is itself able to undergo a reduction reaction to reform iodide.

2.3.2 Application

The Karl Fischer coulometric titrator is set up as shown in Figure 2.6 where a test aliquot is drawn from the sample and injected using a 1 ml plastic syringe through the septum. The coulometric titration commences and a digital readout is generated upon reaching the end point. Readings are given in units of parts per million (ppm). From section 2.3.1, 1 mg of water is equivalent to 10.72 C. Calculations are performed based on the density of solvent used. In this study, mainly acetonitrile ($786\ \text{mg ml}^{-1}$) was used to perform the experiments. In some circumstances, reaction between I_2 and H_2O is not 1:1 and charge measured does not accurately reflect the water content of the test samples due to the presence of other oxidizable species. The present study uses a simple electrolyte and organic solvent mixture which do not contribute additional difficulty in the calculation of the water content for the titrator. Constant humidity measurements were conducted in a humidity control glove box using a dry nitrogen purge system (Section 2.4.). Chapter 4 and Chapter 6 will discuss the results obtained.

2.3.3 Model and Chemicals

Karl Fischer (KF) titrations were conducted with a Mettler Toledo DL32 coulometer using (Riedel-deHaën) HYDRANAL[®]-Coulomat CG for the cathode

compartment and HYDRANYL[®]-coulomat AG for the anode compartment. The chemicals were all obtained from Sigma-Aldrich.

2.4 Humidity Controlled Chamber

A humidity controlled chamber was used to reduce the humidity to a level which is optimum for experiments where water is involved in the reaction. The Karl-Fischer titrator was placed within the humidity chamber in order to increase the lifetime of the anolyte and catholyte. The chamber controls and sets an ambient humidity level thereby lowering the effect that moisture in the environment might have on the titrator.

Dry nitrogen gas is used to purge the chamber while the automatic controller controls the release of the gas through to the chamber. Once a relative humidity is set, the automatic controller will allow the system to dehumidify or humidify by reading signals from the humidity sensor in the chamber. The humidifier is activated when necessary and a mist is generated via ultrasound vibration of a water supply. This is exchanged with the dry gas in the chamber via a pump system and this makes up a closed loop system where humidity can be maintained with the constant supply and presence of inert gas.

Figure 2.7 below shows a simplified flow diagram of the humidity controlled chamber. The experiments carried out in the chamber (measured 122cm × 61cm × 61cm) were conducted at 30% relative humidity.³⁷ As the mean relative humidity in Singapore is measured at 84%,³⁸ the chamber has to be set at a significantly lower value. Maintaining a level lower than 30% requires a long time to achieve and is easily affected by moisture when hands enter from the gloveless sleeves.

Figure 2.7: Humidity controlled chamber flow diagram.

2.5 Spectroscopic Methods

Spectroscopic methods were used in some instances as quantitative and qualitative measures in addition to the electroanalytical methods used to verify the identity of various intermediates species formed. The approach allows the study to be more holistic and further prove the accuracy of the results obtained via electrochemical methods. Two spectroscopic techniques were used in this thesis; UV-Vis³⁹ and FT-IR^{40,41} spectroscopies.

UV-Vis spectra were recorded with a Perkin-Elmer Lambda 750 spectrophotometer and UV WinLab Software.

FT-IR spectra were obtained with a Shimadzu IRPrestige-21 FTIR spectrophotometer.

2.6 Low Temperature Studies

Performing experiments at low temperatures sometimes allows for electrogenerated reactive compounds to be more long-lived than under ambient conditions.⁴² This helps in the study of the reaction mechanism where short-lived or reactive species formed would otherwise not be noticeable at room temperature.

Variable-temperature experiments included CV scans performed using a Metrohm jacketed glass cell (Figure 2.4) and low temperature coulometry experiments (electrolysis) conducted in a custom jacketed glass cell (Figure 2.3). The temperature was adjusted

when needed and a steady temperature was achieved when the rate of warming the cell from the surrounding equals the rate of cooling by the low temperature circulator.⁴³ During the lowering of temperature, Ar gas was constantly purged through the solution to ensure the environment within the cell remained free of oxygen. This also reduced the amount of water from the surroundings entering the cell during the cooling process. Conducting experiments at a low temperature increases the resistance and viscosity of the solvent system. Similar experiments were conducted with just the solvent system without the analyte to obtain the background current. Analysis and digital simulation of data were performed after the background correction.⁴⁴

The temperature was controlled with a Julabo FP89-HL ultralow refrigerated circulator which uses ethanol as the internal circulating solution. An additional Thermo Electron Neslab RTE 740 circulating bath with an internal circulating solution of isopropanol was used to control a different cell concurrently.

2.7 Preparation for Ultra-Dry Experimental Set Up

As electrochemical experiments with accurately controlled water concentrations are difficult to perform due to the many ways that water can enter the cell, careful calibration of the experiments were required.⁴⁵ In Chapter 4 and 6, a special experimental set up was performed in order to prepare the ultra-dry conditions.

To prevent moisture present in the reagents from affecting the experimental results, drying of the solvents and electrolyte was carried out prior to the experiment. Fresh molecular sieves were heated to 413 K for 6 h and added to the solvent in the solvent bottle and left to stand for 24 h with occasional swirling to facilitate the trapping of water molecules.^{37,46,47} The glass solvent bottle was preheated at 373 K for 2 h while the plastic cap pre-warmed at 323 K. Karl-Fischer coulometric titrations (Section 2.3) were carried out on the solvent to determine the water content before and after drying.

0.2 M supporting electrolyte (Bu_4NPF_6) was weighed into a 25 ml volumetric flask and heated at 413 K for 6 h in a vacuum oven. At the end of heating, the electrolyte was dissolved with 25 ml of dried solvent from the solvent bottle and the solvent/electrolyte mixture poured into the pre-dried vacuum syringe (containing molecular sieves) and kept under a constant flow of nitrogen gas for 2 days before being used. The vacuum syringe was heated at 373 K for 2 h prior to use. 0.5 M supporting electrolyte (Bu_4NPF_6) was weighed into a 5 ml volumetric flask and dissolved with dry solvent from the solvent bottle to make up the internal filling solution of an Ag wire reference electrode.

A control electrochemical cell containing just the solvent/electrolyte was prepared to estimate the amount of water entering the electrochemical cell over time under the natural humidity conditions. All drying procedures were prepared in the humidity controlled chamber to lower the moisture contamination from the environment (Section 2.4).

2.8 Light Sensitive Experiment

All compounds were prepared under dim lights in the laboratory and glassware were wrapped with foil to prevent photodecomposition. During the experiment itself, lights remained dim throughout the entire process.

2.9 General Experimental Section

2.9.1 Chemicals and Solvents

The experiments performed and discussed in this thesis used either HPLC or analytical grade acetonitrile (CH_3CN) obtained from (ACI labscan) and (Tedia) respectively, or analytical grade dichloromethane (CH_2Cl_2) obtained from (Tedia) as the solvent to dissolve the compound and electrolyte. Dichloromethane is specially used to dissolve β -carotene in experiments conducted as it is not soluble in acetonitrile. Molecular

sieves of the form $\frac{1}{16}$ in. rods with 3 Å pore size (CAS 308080-99-1) were obtained from Fluka.

In Chapter 3, β -carotene (>97%) was obtained from TCI Japan, all-trans-retinol was obtained from Acros Organics, all-trans-retinal was obtained from Sigma-Aldrich, retinyl palmitate was obtained from Supelco Analytical, retinoic acid was obtained from Alfa Aesar, retinyl acetate was obtained from Sigma-Aldrich and they were all stored in the dark at 277 K. Acetonitrile and dichloromethane was used directly from the bottles.

In Chapter 4, all trans-retinal ($\leq 98\%$) was obtained from Sigma-Aldrich and stored in the dark at 277 K. Acetonitrile was used directly from bottles for the normal experimental conditions. During the dry solvent experiments, acetonitrile was used after drying over 3 Å molecular sieves (Section 2.7). For the addition of water to the solution, purified water, with a resistivity of $\geq 18 \text{ M}\Omega \text{ cm}$ was obtained from an ELGA Purelab Option-Q system.

In Chapter 5, ($\pm$)- α -tocopherol (α -TOH) (97%) was obtained from Sigma-Aldrich and stored in the dark under vacuum and β -carotene (>97%) was obtained from TCI Japan and stored in the dark at 277 K. Nitrosonium hexafluoroantimonate (NOSbF_6) (99%) used for chemical oxidation was obtained from Sigma-Aldrich and stored in a glovebox under a nitrogen atmosphere. Acetonitrile and dichloromethane were used directly from the bottles (after drying).

In Chapter 6, ($\pm$)- α -tocopherol (α -TOH) (97%) was obtained from Sigma-Aldrich and stored in the dark under vacuum. During the dry solvent experiments, acetonitrile was used after drying over 3 Å molecular sieves (Section 2.7). For the addition of water to the solution, purified water, with a resistivity of $\geq 18 \text{ M}\Omega \text{ cm}$ obtained from an ELGA Purelab Option-Q system was used.

2.9.2 Electrolyte

In all the experiments conducted in this thesis, tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) was used as the supporting electrolyte. The solubility of Bu_4NPF_6 in organic solvent is generally high due to its bulky cation and anion⁴⁵ and it is possible for the solvent system of Bu_4NPF_6 and acetonitrile to be used over a wide potential range.⁴⁸

Bu_4NPF_6 was prepared by reacting equal molar amounts of aqueous solutions of Bu_4NOH (40% Alfa Aesar) and HPF_6 (65%, Fluka), washing the precipitate with hot water, and recrystallizing 3 times from hot ethanol followed by drying under vacuum at 433 K for 24 h and storing under vacuum before its use.

2.9.3 Theoretical Calculations

Digital modelling studies to obtain kinetic and equilibrium parameters for the reaction mechanisms of vitamins A and E were performed using the DigiElch 7.F software package.⁴⁹⁻⁵⁴

2.10 References

1. Heineman, W. R.; Kissinger, P. T. *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Marcel Dekker: New York, 1996, 84-85.
2. Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd Ed.; Wiley, New York, 2001, 239-247.
3. Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd Ed.; Wiley, New York, 2001, 487-516.
4. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 59-68.
5. Monk, P. M. S. *Fundamentals of Electroanalytical Chemistry*, Wiley: England, 2001, 156-162.
6. Zachowski, E. J.; Wojciechowski, M.; Osteryoung, J. *Anal. Chim. Acta.* **1986**, 183, 47-57.
7. Osteryoung, J. G.; Osteryoung, R. A. *Anal. Chem.* **1985**, 57, 101A-110A.
8. Christie, J. H.; Turner, J. A.; Osteryoung, R. A. *Anal. Chem.* **1977**, 49, 1899-1903.
9. Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd Ed.; Wiley, New York, 2001, 293-299.
10. Thomas, F. G.; Henze, G. *Introduction to Voltammetric Analysis: Theory and Practice*, CSIRO: Collingwood VIC, 2001, 39-43.
11. Monk, P. M. S. *Fundamentals of Electroanalytical Chemistry*, Wiley: England, 2001, 182-185.
12. Monk, P. M. S. *Fundamentals of Electroanalytical Chemistry*, Wiley: England, 2001, 195-209.

13. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 69-71.
14. Fisher, A. C. *Oxford Chemistry Primers, Electrode Dynamics*, Oxford University Press: Oxford, 1996, 27-31.
15. Lovrić, M.; Osteryoung, J. J. *Electroanal. Chem.* **1986**, 197, 63-75.
16. Fisher, A. C. *Oxford Chemistry Primers, Electrode Dynamics*, Oxford University Press: Oxford, 1996, 20-22.
17. Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd Ed.; Wiley, New York, 2001, 335-348.
18. Monk, P. M. S. *Fundamentals of Electroanalytical Chemistry*, Wiley: England, 2001, 136-141.
19. Fisher, A. C. *Oxford Chemistry Primers, Electrode Dynamics*, Oxford University Press: Oxford, 1996, 32-33.
20. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 102-104.
21. Inzelt, G. In *Electroanalytical Methods: Guide to Experiments and Applications*, Scholz, F., Eds.; Springer-Verlag Berlin Heidelberg: Berlin, Heidelberg, 2010, 147-158.
22. Kissinger, P. T.; Heineman, W. R. *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Marcel Dekker, Inc.: New York, 1996, 55-60.
23. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 36-40.
24. Gritzner, G.; Kuta, J. *Pure Appl. Chem.* **1984**, 56, 461-466.

25. Connelly, N. G.; Geiger, W. E. *Chem. Rev.* **1996**, 96, 877-910.
26. Cardwell, T. J.; Mocak, J.; Santos, J. H.; Bond, A. M. *Analyst* **1996**, 121, 357-362.
27. Fischer, K. *Angew. Chem.* **1935**, 48, 394-396.
28. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R. *Fundamentals of Analytical Chemistry*, 8th Ed.; Brooks/Cole- Thomson Learning: U.S.A, 2004, 580-582.
29. Curran, D. J. In *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Kissinger, P. T.; Heineman, W. R., Eds.; Marcel Dekker: New York, 1996, 757.
30. Verhoef, J. C.; Barendrecht, E. *Anal. Chim. Acta.* **1977**, 94, 395-403.
31. Verhoef, J. C.; Barendrecht, E. *J. Electroanal. Chem.* **1976**, 71, 305-315.
32. Verhoef, J. C.; Barendrecht, E. *J. Electroanal. Chem.* **1976**, 75, 705-717.
33. Verhoef, J. C.; Barendrecht, E. *J. Electroanal. Chem.* **1978**, 86, 407-415.
34. Verhoef, J. C.; Barendrecht, E. *J. Electroanal. Chem.* **1978**, 93, 75-80.
35. Scholz, E. *Karl Fischer Titration*, Springer-Verlag: Berlin, 1984.
36. Mettler Toledo *Fundamentals of the Coulometric Karl Fischer Titration with Selected Applications*, Gordon, C.; Rohner, R., Eds.; Mettler-Toledo.
37. Hui, Y.; Webster, R. D. *Anal. Chem.* **2011**, 83, 976-981.
38. Local Climatology: Climatology of Singapore: Relative Humidity. National Environmental Agency Web Site. <http://app2.nea.gov.sg/weather-climate/climate-information/local-climatology> (accessed June 6th, 2013).
39. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R. *Fundamentals of Analytical Chemistry*, 8th Ed.; Brooks/Cole- Thomson Learning: U.S.A, 2004, 718-723.
40. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 113.

41. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R. *Fundamentals of Analytical Chemistry*, 8th Ed.; Brooks/Cole- Thomson Learning: U.S.A, 2004, 775-781.
42. Bond, A. M. *Broadening Electrochemical Horizons: Principles and Illustration of Voltammetric and Related Technique*, Oxford University Press: New York, 2002, 208.
43. Evans, D. H.; Lerke, S. A. In *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Kissinger, P. T.; Heineman, W. R., Eds.; Marcel Dekker: New York, 1996, 487-510.
44. Bowyer, W. J.; Engelman, E. E.; Evans, D. H. *J. Electroanal. Chem.* **1989**, 262, 67-82.
45. Fry, A. J. In *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Kissinger, P. T.; Heineman, W. R., Eds.; Marcel Dekker: New York, 1996, 469-485.
46. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D. *J. Am. Chem. Soc.* **2009**, 131, 1523-1534.
47. Hui, Y.; Chng, E. L. K.; Chua, L. P.-L.; Liu, W. Z.; Webster, R. D. *Anal. Chem.* **2010**, 82, 1928-1934.
48. Fleischmann, M.; Pletcher, D. *Tetrahedron Lett.* **1968**, 60, 6255-6258.
49. Rudolph, M. *J. Electroanal. Chem.* **2003**, 543, 23-39.
50. Rudolph, M. *J. Electroanal. Chem.* **2003**, 558, 171-176.
51. Rudolph, M. *J. Electroanal. Chem.* **2004**, 571, 289-307.
52. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 619-632.
53. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 633-641.
54. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 1193-1204.

Chapter 3

The Differences in Voltammetric Behavior of Different forms of Vitamin A in Aprotic Organic Solvents

3.1 Introduction

Being a liposoluble vitamin (Scheme 3.1), vitamin A is naturally absorbed and stored in the fat cells and this is where the various individual biochemical reactions of interest occur. In this study, aprotic organic solvents CH_3CN and CH_2Cl_2 were employed to study the redox reactions in an environment that has some similarities to the lipophilic environment where the different species of vitamin A reside.^{1,2}

Scheme 3.1: Structures of vitamin A derivatives. (1) All-trans-β-carotene. (2) Retinoic acid. (3) All-trans-Retinol. (4) Retinyl palmitate. (5) All-trans-Retinyl acetate. (6) All-trans-Retinal.

A number of studies have suggested the carotenoids convert to retinoids via enzymatic biological cleavage³⁻⁵ while other studies have concluded that they do not metabolize to one another.⁶⁻⁸ It is interesting to see how the different species of vitamin A might be interlinked with one another while undergoing different biochemical reactions via varying reaction pathways (Scheme 3.2).

Scheme 3.2: Bioconversion and redox mechanism of carotenoids and retinoids obtained from different sources of food.

One common association between biological activity and electrochemical reactions is that they involve electron transfer or oxidation and reduction reactions resulting in conversions between the different species (Scheme 3.2).⁹ Electron transfer reactions of vitamin A compounds can be examined with the use of electrochemical methods. A series of cyclic voltammetry (CV) experiments were conducted on the six different derivatives of vitamin A. Each of the compounds responded differently by showing different oxidation and reduction processes under the same experimental

conditions. This is interesting because not all vitamin A species behave and react the same way biochemically in the body and this could be an explanation for the different role each form may partake. In this Chapter, we use electrochemistry and controlled potential electrolysis with coulometry measurements to study the difference in voltammetric behavior of different vitamin A species in aprotic solvents.

3.2 Reductive Cyclic Voltammetry of Different Forms of Vitamin A in CH₃CN and CH₂Cl₂ using GC and Pt Working Electrodes

The different forms of vitamin A showed varying voltammetric responses when tested under the same experimental conditions. Cyclic voltammograms performed with a 1 mm diameter GC electrode in CH₃CN at a scan rate of 0.1 V s⁻¹ for retinal (**6**), retinyl acetate (**5**), retinyl palmitate (**4**), retinoic acid (**2**) and retinol (**3**) showed two reductive peaks, while the reductive peaks observed for retinol (**3**) had surprisingly very small peak currents as shown in Figure 3.1 (Solid lines). A reduction scan (dotted line) for (**2**), (**4**), (**5**) and (**6**) was conducted up to the first reduction peak to examine the chemical reversibility of the first reduction process. All reduction processes were found to be not chemically reversible at a scan rate of 0.1 V s⁻¹ except for the first reduction peak of retinal. The interesting reduction behavior of retinal has been studied more thoroughly and has been shown to undergo a series of hydrogen-bonding and dimerization reactions (which is described in considerable detail in Chapter 4).¹⁰

Retinal (**6**) had the lowest reduction peak potential of -1.78 V vs. Fc/Fc⁺ (Fc=ferrocene) followed by retinoic acid (-1.90 V vs. Fc/Fc⁺) (**2**), retinyl palmitate (-2.14 V vs. Fc/Fc⁺) (**4**) and retinyl acetate (-2.27 V vs. Fc/Fc⁺) (**5**). The peak currents obtained during the reduction of retinol (**3**) were very small compared to the rest of the retinoids tested, which seems unusual because (**3**) is believed to be one of the most important forms of vitamin A.¹¹⁻¹³ The reason for the small peak currents for (**3**) does not arise from a

much smaller diffusion coefficient, since it is of similar size to the other compounds. Instead the difference may arise from chemical instability in solution. Different sources of retinol were examined but similar electrochemical responses were obtained in each case, with retinol displaying relatively small peak currents compared to the other compounds.

Figure 3.1: CVs of 1×10^{-3} M retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at a scan rate of 0.1 V s^{-1} (black solid line) scan up to the second reduction peak and (black dotted line) upon reaching the first reduction peak.

Compounds (**2**), (**4**), (**5**) and (**6**) have very different responses in terms of the separation between the first and second reduction peak potentials (Table 3.1). (**2**) registers the largest peak potential separation between the first reduction process and the second, while (**6**) displays the smallest potential separation.¹⁰ However, the two reduction peaks do not represent the same processes for each compound. For retinal (**6**), the two electron transfer steps are associated with the one-electron (eq 3.1) and then second electron transfer (eq 3.2) to form the anion radical and then the dianion, respectively. However,

the other compounds display a chemically irreversible first reduction process, thus the second reduction process does not simply involve the further one-electron reduction of the anion radical to form the dianion, but instead is associated with the further reduction of a reaction product of the first electron transfer.

Compound	1st reduction peak potential (V vs. Fc/Fc ⁺)	2nd reduction peak potential (V vs. Fc/Fc ⁺)	peak separation/V
(1) β -carotene	-2.10	-	-
(2) Retinoic Acid	-1.90	-2.50	0.60
(3) Retinol	-2.56	-2.74	0.18
(4) Retinyl Palmitate	-2.14	-2.66	0.52
(5) Retinyl Acetate	-2.27	-2.72	0.45
(6) Retinal	-1.78	-2.09	0.31

Table 3.1: Reduction peak potentials of 1×10^{-3} M of different vitamin A compounds obtained in CH₃CN using a 1 mm diameter GC working electrode at 295 ± 2 K with a scan rate of 0.1 V s^{-1} . β -Carotene was measured in CH₂Cl₂.

β -carotene, which is one of the most abundant forms of the carotenoids in the human body,¹⁴ is insoluble in CH₃CN¹⁵ hence another set of experiments were performed to enable a comparison of the carotenoid with the retinoids under the same experimental conditions. The experiments were conducted using CH₂Cl₂ as the solvent. When the experiments were performed in CH₂Cl₂ using a 1 mm GC working electrode, compound (2) and (6) showed two reductive peaks, whereas only one reductive peak was observed for (1) and (5). The other compounds ((3) and (4)), do not have any detectable reduction response (Figure 3.2).

Figure 3.2: CVs of 1×10^{-3} M β -carotene (**1**), retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at a scan rate of 0.1 V s^{-1} ; (black solid line) scan up to the second reduction peak and (black dotted line) upon reaching the first reduction peak.

Using a different electrode in CH_3CN resulted in very different electrochemical responses for the retinoids. Of all the retinoids, only (**2**) and (**6**) were found to undergo a detectable reduction reaction on a Pt electrode (Figure 3.3). Compound (**2**) appears significantly more easily reduced than (**6**) when the working electrode is changed from GC to Pt. Furthermore, on Pt, (**2**) displays one apparently chemically reversibly reduction process (but with a wide separation between the forward and reverse peaks) at -1.35 V vs. Fc/Fc^+ , instead of two reduction peaks as observed on GC. The electrochemical response of (**6**) on Pt is very similar to that observed on GC where two reduction processes are observed, although the first reduction process shows less chemical reversibility on Pt. All other retinoids did not show a reduction response within the potential window that is available with the Pt electrode.

Figure 3.3: CVs of 1×10^{-3} M retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode at a scan rate of 0.1 V s^{-1} ; (black solid line) scan up to the second reduction peak and (black dotted line) upon reaching the first reduction peak.

When switching the working electrode to Pt, only compounds (**2**) and (**6**) display reduction responses in CH_2Cl_2 (Figure 3.4). This is very similar to the result obtained for the Pt electrode when experiments were performed in CH_3CN . The peak currents obtained for the reduction processes for all compounds were higher in CH_3CN compared to CH_2Cl_2 and higher on a GC electrode compared to Pt.

Figure 3.4: CVs of 1×10^{-3} M β -carotene (**1**), retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode at a scan rate of 0.1 V s^{-1} in an initial reductive direction.

3.2.1 Reductive Cyclic Voltammetry of β -carotene (**1**)

The reduction process of (**1**) can be observed as a single cathodic peak when using a 1 mm diameter GC WE. The cathodic peak is not chemically reversible at a slow scan rates, however, as the scan rate increases, it starts to show signs of reversibility (Figure 3.5). The reduction process constitutes a $2e^-$ transfer, which was estimated by comparison with the peak current of the oxidation process (and from the coulometry experiments presented later in the Chapter). This is different from the result previously obtained by Mairanovsky¹⁶ and Park¹⁷ who stated a $1e^-$ process for the first reduction peak using dimethylformamide (DMF) and tetrahydrofuran (THF), respectively as the solvents. However, they detected an additional reduction process after the first reduction,^{16,17} and it is possible that under the present experimental conditions, the multiple electron transfer

process is merged into one single peak. A dianion is likely initially formed by the two-electron reduction and it undergoes protonation with any trace water present in the solvent used.¹⁶ Nevertheless, there are no additional peaks found on the reverse scan which might correspond to the oxidation of the chemically converted reduced product.

Figure 3.5: CVs of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rate in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

Similar CV experiments were performed using a 1 mm Pt WE. However, it was not possible to clearly see the reduction peak generated due to the potential limit of the Pt surface reaction (Figure 3.6). The reduction peak obtained is very similar to the background peak obtained at a Pt electrode surface using DMF as solvent with Et_4NBr as electrolyte.¹⁸

Variable concentration experiments performed on the reduction of (**1**) at a Pt WE showed an obvious increase in the peak at -0.85 V vs. Fc/Fc^+ (Figure 3.7), which does

correspond to the reduction of the analyte rather than a background response. This indicates that the analyte peak is likely merging with the background peak, and it is difficult to tell the peaks apart.

Figure 3.6: CVs of $1 \times 10^{-3} \text{ M } \beta$ -carotene (**1**) in CH_2Cl_2 containing $0.2 \text{ M Bu}_4\text{NPF}_6$ at $295 \pm 2 \text{ K}$ at a 1 mm diameter Pt working electrode with varying scan rate in an initial reductive direction. The current has been normalized by multiplying by $v^{-0.5}$ where v is the scan rate.

Figure 3.7: CVs of 2.0×10^{-3} M, 1.0×10^{-3} M and 0.5×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode at 0.5 V s^{-1} in an initial reductive direction.

3.2.2 Reductive Cyclic Voltammetry of Retinoic Acid (**2**)

Variable scan rate CV experiments were performed on compound (**2**) and it was found that the first cathodic peak at $-1.90 \text{ V vs. Fc/Fc}^+$ remains chemically irreversible even at the fastest scan rate used of 10 V s^{-1} . There were also no significant changes to the second cathodic peak, where it also remains chemically irreversible at higher scan rates. At a higher concentration of $2 \times 10^{-3} \text{ M}$, the second cathodic peak was observed to be significantly larger compared to the first cathodic peak (Figure 3.8). Comparing this to the CV obtained of $1 \times 10^{-3} \text{ M}$ (Figure 3.1), the first cathodic peak appears approximately half the peak height of the second cathodic peak. In addition, electrolysis with the potential held just after the first reduction peak, produced a product that was reduced at the potential of the second reduction peak and with a substantially larger peak current than that of retinoic acid (Figure 3.39). This indicates that the second reduction process is due to a reaction product produced in the first electron transfer process (probably via an EC mechanism), and that the second electron transfer reaction (of retinoic acid) involves the transfer of more electrons than the first process. Theoretically, (**2**) will be reduced to (**6**) via a chemical reduction process. This is interesting because biologically, retinoic acid

(2) is being converted from retinal (6) and it might be possible for retinoic acid (2) to be reduced back to retinal (6), whenever necessary by the body.

Figure 3.8: CVs of 2×10^{-3} M retinoic acid (2) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rate in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

Reduction of (2) when performed using Pt WE interestingly showed an apparent chemically reversible first reduction process, but which broadens and appears to become less chemically reversible as the scan rate increases (Figure 3.9). The result suggests that (2) undergoes relatively slow heterogeneous electron transfer at the Pt surface, which accounts for the widening peak separation at fast scan rates. On a Pt WE, the second reduction process cannot be detected, as it occurs outside the potential window available on Pt. However, it is uncertain whether the second electron transfer step would occur at all on Pt, since it has been shown to arise on a GC electrode due to a product of the initial reduction (but on Pt the initial reduction appears chemically reversible, so a reaction

product is not necessarily expected). Coulometry at a Pt electrode showed that the first reduction peak corresponded to a $1e^-$ transfer, which is discussed in section 3.5.2.

Figure 3.9: CVs of 2×10^{-3} M retinoic acid (**2**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode with varying scan rates in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.2.3 Reductive Cyclic Voltammetry of Retinol (**3**)

The reduction response of (**3**) was also different in CH_3CN and CH_2Cl_2 . On the same electrode surface (GC), (**3**) showed two reduction peaks when CH_3CN was used while only one reduction peak was seen when CH_2Cl_2 was used (Figure 3.10). In CH_3CN , the two peaks occur at very negative potentials and were of similar size to the peak current observed for the oxidation process. No reduction peak was visible on the CV scan conducted on 1 mm Pt WE in both solvents used (Figure 3.2 and 3.4). At this time the reason for the very small peak current is unclear, but it is likely to be from chemical

instability of the compound in the solvents used, rather than an electrochemical effect (such as low diffusion coefficient or low number of electrons transferred).

Figure 3.10: CVs of 1×10^{-3} M retinol (**3**) in (a) CH_3CN and (b) CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with 0.1 V s^{-1} scan rate in an initial oxidative direction.

3.2.4 Reductive Cyclic Voltammetry of Retinyl Palmitate (**4**)

Retinyl palmitate can be electrochemically reduced in two processes at very negative potentials on a GC WE. As the scan rate increased, the reduction processes of retinyl palmitate (**4**) showed signs of chemical reversibility. This is the case for both the first and second cathodic peaks (Figure 3.11). Increasing scan rates shifted the potential of the first reduction peak more negatively, hence closing the potential gap between the first and second reduction peaks of (**4**). On the same electrode but with the different solvent CH_2Cl_2 , the two reduction processes were not observed. However, when an experiment was conducted at a lower temperature of 253 K in CH_2Cl_2 , a single cathodic peak was seen (Figure 3.12). Lowering the temperature possibly reduces the background current in GC, sufficient to make the signal of the analyte detectable. At faster scan rates in CH_2Cl_2 at 253 K, the first reduction process becomes more chemically reversible.

Figure 3.11: CVs of 1×10^{-3} M retinyl palmitate (**4**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rate in an initial reductive direction. (a) Scanning up to the second reduction peak and, (b) scanning up till the first reduction peak. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

Figure 3.12: CVs of 1×10^{-3} M retinyl palmitate (**4**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC working electrode with varying scan rate in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.2.5 Reductive Cyclic Voltammetry of Retinyl Acetate (**5**)

Retinyl acetate (**5**) shows two very clear reduction peaks when CV experiments were performed in CH_3CN using a 1 mm GC WE. Both reduction peaks do not show signs of chemical reversibility even at scan rates of 10 V s^{-1} . At faster scan rates, a new anodic peak is detected at $\sim -0.50 \text{ V vs. Fc/Fc}^+$ which is more clearly seen if the scan is first extended past the second reduction process (Figure 3.13).

Figure 3.13: CVs of 2×10^{-3} M retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rates in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

When CH_2Cl_2 is used as a solvent, a single voltammetric reduction peak is detected at a scan rate of 0.1 V s^{-1} . A second reduction process becomes apparent at more positive potentials when the scan rate increases (Figure 3.14). The additional cathodic process even appears chemically reversible when the scan rate is large (10 V s^{-1}), but is much smaller than the other reduction process. The large increase in size of the additional (more positive) reduction process as the scan rate increases could be due to a surface confined species (adsorption), where the peak current typically increases in size directly proportionally with the voltammetric scan rate.

Figure 3.14: CVs of 1×10^{-3} M retinyl acetate (**5**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC working electrode with varying scan rates in an initial reductive direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

The reduction processes of (**5**) were not seen when using a 1 mm Pt WE in CH_3CN at room temperature or at 253 K, due to a lower potential window available on Pt. The lack of a response on Pt is likely caused by the limited potential window because of the hydrogen evolution reaction (due to the reduction of trace water).

3.2.6 Reductive Cyclic Voltammetry of retinal (**6**)

The reductive behavior of (**6**) is different from the other vitamers because chemically reversible reduction processes are observed (Figure 3.15). More detailed electrochemical studies were conducted on retinal (**6**) as it is the only vitamer which displays chemically reversible reduction peaks. Being chemically reversible, its reaction

mechanism can be studied with the use of experimental variables such as scan rates, temperature and concentration. The results are discussed in more detail in Chapter 4.

Figure 3.15: CVs of 2×10^{-3} M retinal (**6**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rates in an initial reductive direction. (a) Scanning up to the second reduction peak and, (b) scanning up until the first reduction peak. The current has been normalized by multiplying by $\nu^{0.5}$ where ν is the scan rate.

3.3 Oxidative Cyclic Voltammetry of Different Forms of Vitamin A in CH_3CN and CH_2Cl_2 using GC and Pt Working Electrodes

Electrochemical oxidation of (**1**) – (**6**) yielded a single oxidation peak in both solvents regardless of the electrode surface used (Figures 3.16 – 3.19). (**1**) is the only compound which shows a chemically reversible oxidation peak.¹⁵ Compound (**1**) also registered the lowest oxidation potential, which is expected for the carotenoids which have a longer polyene chain compared to retinoids.¹⁹ Compounds (**2**) and (**6**) have higher

oxidation potential than the other compounds. For all the different conditions tested, **(3)** produced an unexpectedly small current (oxidative and reductive) which suggests that it does not undergo electrochemical oxidation smoothly, in contrast to some studies which had studied its mechanism in isolation of the other compounds.^{11,20-22}

Figure 3.16: CVs of 1×10^{-3} M retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at a scan rate of 0.1 V s^{-1} in an initial oxidative direction.

Considering that all compounds were studied with the same concentration of 1 mM and prepared via dilution methods to minimize error in the concentration of each compound, the small peak current suggests **(3)** behaves differently than the other compounds, with two possible explanations. One possibility is that the oxidation/reduction of **(3)** involves substantially less electrons than the other compounds. This could occur if the oxidized/reduced forms reacted quickly with the starting material to generate species that are not electroactive. Another more likely explanation is that **(3)**

is decomposing or aggregating in solution and so only a small fraction of the starting material is detected in the CVs.²³ The actual number of electrons involved in both the oxidation and reduction processes measured via coulometry experiments will be discussed later in the Chapter.

Figure 3.17: CVs of 1×10^{-3} M β -carotene (**1**), retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at a scan rate of 0.1 V s^{-1} scanning in an initial oxidative direction.

Figure 3.18: CVs of 1×10^{-3} M retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode at a scan rate of 0.1 V s^{-1} in an initial oxidative direction.

Figure 3.19: CVs of 1×10^{-3} M β -carotene (**1**), retinoic acid (**2**), retinol (**3**), retinyl palmitate (**4**), retinyl acetate (**5**) and retinal (**6**) (from top to bottom) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode at a scan rate of 0.1 V s^{-1} in an initial oxidative direction.

3.3.1 Oxidative Cyclic Voltammetry of β -carotene (**1**)

Varying scan rate CV experiments were performed on (**1**) using both Pt and GC WEs in CH_2Cl_2 . As can be seen from both Figures 3.20 and 3.21, at a slower scan rate of 0.1 V s^{-1} , it is possible to see additional oxidation processes occurring at more positive potentials than the first oxidation peak at $\sim +0.15 \text{ V vs. Fc/Fc}^+$. A reduction peak at $-0.36 \text{ V vs. Fc/Fc}^+$ is also visible at a negative potential (with slow scan rates) which belongs to the reduction of an oxidised product of (**1**). When the scan rate is progressively increased to 10 V s^{-1} , the main oxidation peak at $\sim +0.15 \text{ V vs. Fc/Fc}^+$ becomes more chemically reversible and the subsequent oxidation peaks at more positive potentials become less prominent (along with the reduction peak at $-0.36 \text{ V vs. Fc/Fc}^+$) due to the follow up chemical reactions being outrun at faster scan rates.

Figure 3.20: CVs of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.5 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

The oxidative voltammetry of (**1**) has previously been studied thoroughly because of its importance as a vitamin source in mammals as well as its involvement in biological reactions. Electrochemical studies have shown that the oxidation of (**1**) involves the transfer of $2e^-$ where a dication is generated.^{24,25} The size of the reverse cathodic peak after the initial two-electron oxidation is affected by the scan rate as it determines if the dication undergoes a deprotonation reaction or other possible chemical reactions in an overall EC mechanism. The reaction product gives a new reduction response at negative potentials as shown in both Figures 3.20 and 3.21 at a scan rates 0.1 V s^{-1} . Equations 3.3 – 3.5 give the reaction mechanism of (**1**) that was established in previous studies.^{16,17,24-28} Although the oxidation reaction is written in two steps (eqs 3.3 and 3.4), only one

voltammetric peak is detected because the first and second electron transfer steps occur at very similar potentials.

Figure 3.21: CVs of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.5 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $v^{-0.5}$ where v is the scan rate.

A set of low temperature experiments were performed to test if it was possible to stabilize the dication for long periods of time. At 213 K, the CV scan of (**1**) showed a chemically reversible process, however, the anodic and cathodic peaks were broadened which could be due to the slower rate of heterogeneous electron transfer in the low temperature solution (Figure 3.22). As the temperature increased to 293 K, the anodic and cathodic peak became sharper and a new cathodic peak appeared at -0.27 V vs. Fc/Fc^+ .

The variable temperature CV experiments indicate that it is possible to stabilize the dication at low temperatures long enough to obtain completely chemically reversible voltammograms at a scan rate of 0.1 V s^{-1} .

Figure 3.22: CVs of $1 \times 10^{-3} \text{ M}$ β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at varying temperatures of 293 K, 273 K, 253 K, 233 K and 213 K at a 1 mm diameter Pt working electrode at 0.1 V s^{-1} scan rate in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

Variable concentration experiments were also performed to test for other possible reaction pathways. At a lower concentration of 0.2 mM , the dication generated after the oxidation process reacts faster, hence resulting in a less reversible cathodic peak (Figure 3.23). One possible explanation for the increase in reactivity of the dication at low concentrations could be due to reactions with trace water in the solvent, where at low analyte concentrations there is relatively more water compared to the β -carotene.

Figure 3.23: CVs of 1×10^{-3} M, 0.5×10^{-3} M and 0.2×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.5 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode with 0.1 V s^{-1} scan rate in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.3.2 Oxidative Cyclic Voltammetry of Retinoic acid (**2**)

Varying the scan rate on a 1 mm GC WE did not show any interesting trends for the oxidation of retinoic acid, as there was no changes to the peak potential or chemical reversibility of the oxidation process (Figure 3.24). The same oxidation response was obtained when using a 1 mm Pt WE (Data not shown).

Figure 3.24: CVs of 2×10^{-3} M retinoic acid (**2**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.3.3 Oxidative Cyclic Voltammetry of Retinol (**3**)

The peak currents observed during the oxidation and reduction of retinol (**3**) are much lower than compared to the rest of the compounds when experiments were performed under the same experimental conditions. The oxidative peak current for (**3**) is small when conducting experiments using both 1 mm Pt and GC WEs. The oxidative behavior was investigated to see if varying the scan rate made a difference to the voltammetric response.

(**3**) was first examined using 1 mm Pt WE in CH_3CN at a slower scan rate of 0.1 V s^{-1} , and an anodic peak was obtained at $\sim +0.50 \text{ V vs. Fc/Fc}^+$. During the reverse scan, no cathodic peak was seen for the initial oxidised product of (**3**) and it appears to be a

chemically irreversible process. However, upon further scanning the potential in the negative direction (after first oxidizing (**3**)), a cathodic peak appears at ~ -0.40 V vs. Fc/Fc^+ . When the scan rate is increased to 0.5 V s^{-1} , the new cathodic peak at $\sim -0.40 \text{ V s}^{-1}$ shows signs of chemical reversibility, but the reversibility diminishes again as the scan rate reaches 5 V s^{-1} (Figure 3.25).

Figure 3.25: CVs of $1 \times 10^{-3} \text{ M}$ retinol (**3**) in CH_3CN containing 0.2 M Bu_4NPF_6 at $295 \pm 2 \text{ K}$ at a 1 mm diameter Pt working electrode with varying scan rates in an initial oxidative direction where the first scan is represented by black solid line and consecutive scan is represented with a black dotted line. The current has been normalized by multiplying by $v^{-0.5}$ where v is the scan rate.

The CV responses varied when the solvent was changed from CH_3CN to CH_2Cl_2 . The major oxidation peak at $\sim +0.50 \text{ V}$ vs. Fc/Fc^+ is still present in CH_2Cl_2 , but became sharper when compared to the voltammograms recorded in CH_3CN . The cathodic peak that was observed at $\sim -0.40 \text{ V}$ vs. Fc/Fc^+ , due to the oxidised product, remains chemically irreversible even with increasing scan rates (Figure 3.26). Possible oxidative reactions can

be proposed which are similar to molecules which have a conjugated double bond system.^{20,29} The oxidation of **(3)** is also believed to form retinal **(6)** in a $2e^-$ process.¹¹ However, if the anodic peak height were to be used to estimate the number of electrons transferred during the oxidation of **(3)**, by comparing to the other known compound in Figure 3.18, the number of electrons would be $\ll 2$ (closer to 0.3). The actual molecular transformation mechanism will require more studies as the conjugated chain is highly susceptible to cis-trans isomerization which complicates the process.

Figure 3.26: CVs of 1×10^{-3} M retinol **(3)** in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode with varying scan rate in an initial oxidative direction where the first scan is represented by black solid line and the consecutive scan is represented with black dotted line. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.3.4 Oxidative Cyclic Voltammetry of Retinol Palmitate (**4**)

Oxidation of **(4)** at the same electrode surface but with different solvent yields different voltammetric responses. On both electrode surfaces, GC and Pt, a chemically

irreversible oxidation process was detected at +0.50 V vs. Fc/Fc^+ . On a GC electrode in CH_3CN , no reverse reductive peaks were detected when the scan direction is reversed after the first oxidation process (Figure 3.27). However, as can be seen in Figure 3.28, a chemically irreversible reduction process was detected on GC in CH_2Cl_2 at ~ -0.25 V vs. Fc/Fc^+ which is likely to be associated with the reduction of a product of the initial oxidation reaction.

Figure 3.27: CVs of 1×10^{-3} M retinyl palmitate (**4**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

Figure 3.28: CVs of 1×10^{-3} M retinyl palmitate (**4**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

When the voltammetric potential was scanned further towards the negative potential direction after the oxidation process, additional cathodic peaks were seen (Figure 3.29). These additional processes were only seen at 253 K and not at room temperature.

Figure 3.29: CVs of 1×10^{-3} M retinyl palmitate (**4**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at 1 mm diameter GC working electrode at 0.1 V s^{-1} . (a) Scanning in a positive direction followed by a second cycle. (b) Scanning in a negative direction followed by a second cycle. The solid black line represents the first scan while the red dotted line represents the second scan.

3.3.5 Oxidative Cyclic Voltammetry of Retinyl Acetate (**5**)

A single chemically irreversible oxidation peak was detected on a 1 mm GC WE (Figure 3.30). The same chemically irreversible anodic process was obtained at a 1 mm Pt WE in CH_3CN (Figure 3.31). On both electrodes, a product peak was seen when the scan direction was reversed at a potential of $-0.30 \text{ V vs. Fc/Fc}^+$. The presence of a chemically irreversible anodic peak corresponds to a possible EC mechanism, where a chemical product is generated after the initial electrochemical reduction process.

Figure 3.30: CVs of $2 \times 10^{-3} \text{ M}$ retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at $295 \pm 2 \text{ K}$ at a 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $v^{-0.5}$ where v is the scan rate.

Figure 3.31: CVs of 2×10^{-3} M retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

The oxidation process was also studied in CH_2Cl_2 using the GC electrode. On the same GC WE, (**5**) registered no cathodic response in CH_3CN after the compound was first oxidized, while a cathodic peak was seen in CH_2Cl_2 (Figure 3.32).

Figure 3.32: CVs of 1×10^{-3} M retinyl acetate (**5**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC working electrode with varying scan rates in an initial oxidative direction. The current has been normalized by multiplying by $\nu^{-0.5}$ where ν is the scan rate.

3.3.6 Oxidative Cyclic Voltammetry of Retinal (**6**)

A series of consecutive scans was performed on (**6**) at the two different surfaces, GC and Pt, to examine the effect of multiple scans. The results are presented in Figure 3.33. Part (a) shows the CV voltammograms that were obtained after ten consecutive scans on the 1 mm GC WE. The oxidation peak current decreases over time suggesting that the electrode was becoming partially fouled with the product of the oxidation reactions. A very distinct difference is seen when Pt WE was used (Figure 3.33b). The oxidation peak behavior is similar as multiple scans are recorded, with the peak height decreasing due to adsorption effects. However, a set of quasi-reversible cathodic and anodic peaks were seen at a lower potential of ~ -0.38 V vs. Fc/Fc^+ due to a product of

the initial oxidation reaction. Interestingly, the peak current for this new product decreased in size as more scans were performed.

Figure 3.33: CVs of 1×10^{-3} M retinal (**6**) in CH₃CN containing 0.2 M Bu₄NPF₆ at 295 ± 2 K at a (a) 1 mm diameter GC working electrode and (b) 1 mm diameter Pt working electrode with 0.1 V s^{-1} scan rate in an initial oxidative direction. The initial voltammograms are represented by black solid lines, subsequent voltammogram are represented by grey solid lines and final voltammogram are represented by red solid lines.

3.4 Electrolysis of Vitamin A to Determine the number of Electrons Transferred in the Oxidation and Reduction Processes

Controlled potential bulk electrolysis experiments with coulometry were conducted to measure the number of electrons that each of the oxidation and reduction processes of (**1**) to (**6**) involved over long times. From the CV compilation results discussed previously, the best experimental conditions in achieving the optimal oxidation and reduction responses, is when using CH₃CN, except for β -carotene (**1**) which was performed in CH₂Cl₂ due to solubility constraints.

The number of electrons were calculated from the equation:³⁰

$$Q=nNF$$

where

Q = charge, C

n = number of electrons, e⁻/mol

F = Faraday's constant, 96485 C/e⁻

N = Number of moles converted, mol

3.5 Reductive Electrolysis of Vitamin A

3.5.1 Reductive Electrolysis of β -carotene (**1**)

The voltammograms of before and after electrolysis of (**1**) was conducted using GC WE. Pt wire mesh was used for all the bulk electrolysis experiments. Conducting the electrolysis of β -carotene (**1**) at room temperature shows the reduction involved the transfer of 2e⁻ after an exhaustive electrolysis (Figure 3.34).

When conducting the electrolysis at 253 K, it was possible to record up to the transfer of 4e⁻, which suggests that the temperature might have an effect on the reaction of (**1**) when it comes to its oxidation and reduction process.^{15,31} There has been some controversy as to how many electrons are involved in the reduction reaction of (**1**). The earlier studies by Takahashi and Tachi^{32,33} and Kuta and Ju^{34,35} suggested a 4e⁻ reduction process, while Mairanovsky,¹⁶ Bond³⁶ and Park¹⁷ suggested a 1e⁻ process.

Figure 3.34: (a) CV of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the reductive electrolysis of β -carotene at $-2.14 \text{ V vs. Fc/Fc}^+$.

These reported differences might have arisen due to the different experimental conditions that were used as well as the potential that was used for the electrolysis. It was previously stated that the first reduction consisted of a 1e^- process because it was being compared to the peak height of a known oxidation process which was found to be a 2e^- process.¹⁶ However, in the experimental conditions used in this Chapter for (**1**), estimating the peak height from the oxidative current relative to reductive current at the same scan rate does not agree with the assumption of a 1e^- reduction process (Figure 3.35).

Figure 3.35: CV of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} .

However, it is possible that the reduction process involves closely overlapping individual electron steps, and by varying the experimental conditions the individual steps may be partially separated. For example, when the temperature is lowered to 253 K, the reduction wave is considerably broadened and appears to involve multiple processes. Nevertheless, when the electrolysis was carried out at 253 K with the potential held at $\sim -2.10 \text{ V vs. Fc/Fc}^+$, the electrolysis appeared to involve the transfer of two-electrons (Figure 3.36).

Figure 3.36: CV of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter Pt WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell.

Further square-wave voltammetry (SWV) experiments were performed at several temperatures to see if the reduction wave could be differentiated into individual processes. It is apparent that the stepwise process is visible when the SWVs are conducted at 213 K and the peaks slowly merge up to one single response as the temperature increases to 295 ± 2 K (Figure 3.37). This again shows that temperature might be a factor in achieving the accurate reduction response of (**1**).

Figure 3.37: SWVs of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 213 K (Black solid line), 253 K (Red solid line) and 295 ± 2 K (Blue solid line) at a 1 mm diameter Pt WE.

Electrolysis was also conducted to a point where $1e^-$ is reached by applying ~ -2.00 V vs. Fc/Fc^+ at a temperature of 253 K (Figure 3.38). A small product peak at -0.98 V vs. Fc/Fc^+ was observed which could possibly correspond to the oxidation of the dimerized product which has been suggested as a possible reduction reaction mechanism of (**1**).¹⁶

Figure 3.38: CV of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter Pt WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell.

More experiments will need to be done in order to decipher the reduction mechanism of (**1**) fully, as due to the closely overlapping reduction processes it is difficult to distinguish between the different types of chemical process that are occurring by using electrochemical methods alone.

3.5.2 Reductive Electrolysis of Retinoic Acid (**2**)

Two processes are detected during the reduction of (**2**) using CV and a 1 mm GC WE, whereas only one reduction process is detected on Pt. The electrolysis potential was held at -1.90 V vs. Fc/Fc^+ to test the number of electrons transferred during the first broad reduction process, and it was found to be a $1e^-$ step. A CV conducted after the

electrolysis showed that the second reduction peak had grown from a peak current of 3.21 μA at the beginning of electrolysis to 9.32 μA at the end of the 1e^- reduction process (Figure 3.39). Therefore, it is clear that the product of the one-electron reduction itself undergoes reduction in another multi-electron process.

Figure 3.39: (a) CV of 2×10^{-3} M retinoic acid (**2**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 283 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the reductive electrolysis of retinoic acid at $-1.90 \text{ V vs. Fc/Fc}^+$.

The colour of the solution was observed to change from pale yellow to colourless after the 1e^- reduction process (Diagram 3.1). The long term products of the reduction of retinoic acid (**2**) are currently not known.

Diagram 3.1: Electrolysis of retinoic acid (**2**). (a) Initial pale yellow colour solution. (b) After $1e^-$ reduction process, pale yellow solution turn colourless.

Voltammogram of retinoic acid (**2**) on 1 mm Pt WE after the $1e^-$ reduction process shows no sign of new product compared to GC WE (Figure 3.40). The product of the one-electron reduction is not visible within the potential window of 1 mm Pt WE.

Figure 3.40: CV of 1×10^{-3} M retinoic acid (**2**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell.

3.5.3 Reductive Electrolysis of Retinol (**3**)

Electrolysis experiments on retinol were not performed because of the very small reduction peaks seen on both electrode surfaces during CV experiments.

3.5.4 Reductive Electrolysis of Retinyl Palmitate (**4**)

Reductive electrolysis was performed on compound (**4**) and the reaction was stopped after the transfer of one-electron per molecule (Figure 3.41). A CV scan was conducted after the electrolysis and it was found that the peak current of the first reductive peak at $-2.20 \text{ V vs. Fc/Fc}^+$ had decreased to half the size of the CV of the starting material (**4**). The second reduction peak at more negative potentials appeared to be of a similar size as prior to the electrolysis.

Figure 3.41: CV of 1×10^{-3} M retinyl palmitate (**4**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell.

The electrolysis reaction was also performed by allowing it to reach its completion, which resulted in the transfer of $2e^-$ per molecule. The CV performed at the completion of the electrolysis showed that the first reduction peak had completely disappeared while the second reduction peak remained the same size as prior to the electrolysis (Figure 3.42). Overall, it appears that the long term product of the reduction is the species that is detected at the second reduction process (during CV experiments on the starting material), similar to the result that was obtained for retinoic acid. Therefore, the reaction can either involve the two-electrons reduction to form a dianion which then reacts quickly to form a new product (EEC process), or alternatively there is an initial electron transfer to form a radical anion which reacts quickly to form another compound which is further reduced (in an ECE or ECEC type process).

Figure 3.42: (a) CV of 1×10^{-3} M retinyl palmitate (**4**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the reductive electrolysis of retinyl palmitate $-2.24 \text{ V vs. Fc/Fc}^+$.

Diagram 3.2: Reductive electrolysis of retinyl palmitate (**4**). (a) Initial colourless solution. (b) After $2e^-$ reduction process, colourless solution turn orange brown.

Diagram 3.2 shows how compound (**4**) in solution changes from a colourless starting state to orange colour along the reduction electrolysis pathway.

3.5.5 Reductive Electrolysis of Retinyl Acetate (**5**)

Reductive electrolysis with the potential held slightly more negative than the first reduction peak of (**5**) resulted in the transfer of $2e^-$ per molecule. A CV obtained at the end of the electrolysis showed that the peak associated with the first reduction process was no longer present (Figure 3.43). The second reduction peak remained unchanged,

except for a shoulder that appeared at a slightly more positive potential. A new oxidative peak was detected at a positive potential which presumably corresponds to the oxidation of the generated reduced products.

Figure 3.43: (a) CV of 1×10^{-3} M retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the reductive electrolysis of retinyl acetate at $-2.22 \text{ V vs. Fc/Fc}^+$.

Diagram 3.3: Reductive electrolysis of retinyl acetate (**5**). (a) Initial colourless solution. (b) After reaching $2e^-$ reduction, solution changes to orange.

The colour of the solution during the electrolysis reaction changed from initially colourless to orange at the end of a $2e^-$ bulk electrolysis process (Diagram 3.3). This colour is very similar to the colour obtained during the reductive electrolysis of retinyl palmitate (**4**).

3.5.6 Reductive Electrolysis of Retinal (6)

Reductive electrolysis was performed at 253 K in CH_3CN for (6) and a total of $1e^-$ was achieved at the end of the electrolysis at -1.84 V vs. Fc/Fc^+ (Figure 3.44). In this instance, both the first and second reduction peaks disappeared when a CV was run at the end of the electrolysis and a new oxidized peak appeared at -0.94 V vs. Fc/Fc^+ . The new oxidation peak has been proposed to be from a dimerized product from the radical anion generated after the first reduction.¹⁰

Figure 3.44: (a) CV of 2×10^{-3} M retinal (6) in CH_3CN containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinal at -1.84 V vs. Fc/Fc^+ .

Electrolysis was conducted at a lower temperature in the hope to see any signs of reactive species generated which would otherwise not be visible at room temperature. Diagram 3.4 shows the colour changes from an initial yellow to intense orange at the end of the $1e^-$. The single colour change suggests a straightforward transformation of (6) to its radical anion.

Diagram 3.4: Reductive electrolysis of retinal (**6**) at 253 K. (a) Initial yellow solution. (b) After some time into the reduction, solution turns orange. (c) Finally reaching $1e^-$ reduction, solution changes to dark orange.

3.6 Oxidative Electrolysis of Vitamin A

3.6.1 Oxidative Electrolysis of β -carotene (**1**)

CV experiments performed on (**1**) showed that it undergoes a chemically reversible oxidation process (Figure 3.45a).^{16,24-28} Bulk controlled potential coulometry indicated that the oxidation occurred via $2e^-$ (Figure 3.45b). After an exhaustive electrolysis was performed at 253 K, CV scans were conducted to see if the two-electron oxidized product survived on the electrolysis time scale. The partial survival of the primary oxidized compound ($\mathbf{1}^{2+}$) was partly due to the lower temperature which helped to lengthen its lifetime under electrolysis conditions. New reaction products were also detected which can be oxidized at a higher potential at $\sim +0.55$ V vs. Fc/Fc^+ as well as being reduced at a lower potential of $\sim +0.32$ V vs. Fc/Fc^+ .

Figure 3.45: (a) CV of 1×10^{-3} M β -carotene (**1**) in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of β -carotene at $+0.38 \text{ V vs. Fc/Fc}^+$.

3.6.2 Oxidative Electrolysis of Retinoic Acid (**2**)

The voltammetric oxidation process of retinoic acid (**2**) appears as a single chemically irreversible peak which was found to be a $2e^-$ process via coulometry conducted at $295 \pm 2 \text{ K}$ in CH_3CN . The CV of the reaction solution conducted after the bulk transfer of $2e^-$ shows that the peak at potential $+0.61 \text{ V vs. Fc/Fc}^+$ disappears while a new peak at slightly more positive potential appears. No other peaks were detected for oxidation products. However, further oxidation is possible which would result in the transfer of more than $2e^-$ overall (Figure 3.46).

Figure 3.46: (a) CV of 1×10^{-3} M retinoic acid (**2**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinoic acid at $+0.61 \text{ V vs. Fc/Fc}^+$.

It was observed that colour change of the solution was extreme, starting from a pale yellow to bright pink followed by orange brown and finally greenish yellow by end of the electrolysis (Diagram 3.5). The whole colour changing process took place within the $2e^-$ reduction process. It was found that the bright pink compound was not long-lived, since once the bulk reduction process was stopped at this stage of the electrolysis, the solution quickly turned orange brown. It is possible that the species responsible for the bright pink colour was an intermediate radical cation, which are frequently brightly coloured and short-lived.

Diagram 3.5: Oxidative electrolysis of retinoic acid (**2**). (a) Initial pale yellow colour solution. (b) When reduction process started, pale yellow solution turn bright pink immediately. (c) Starting material totally exhausted and bright pink solution turned orange brown. (d) Finally, upon reaching $2e^-$ oxidation, solution changes to greenish yellow.

The CV conducted at the end of the electrolysis with a 1 mm Pt electrode (rather than GC) showed new products were generated which can be reduced at a lower potential. Two sets of reductive peaks were observed and both sets appear partially chemically reversible. Experiments were repeated by decreasing the scanning range of the CV scan, and it was possible to see a reduction peak at the same lower potential as seen in Figure 3.47a at the end of the $2e^-$ reduction, which is slightly reversible at the CV time scale (Figure 3.47b).

Figure 3.47: CV of 1×10^{-3} M retinoic acid (**2**) in CH₃CN containing 0.2 M Bu₄NPF₆ at 295 ± 2 K at a 1 mm diameter Pt working electrode at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of two electrons per molecule in a controlled potential electrolysis cell with scanning potential up till (a) $-1.87 \text{ V vs. Fc/Fc}^+$ and (b) $-0.87 \text{ V vs. Fc/Fc}^+$.

3.6.3 Oxidative Electrolysis of Retinol (3)

Electrolysis experiments on retinol were not performed because of the very small oxidation peaks seen on both electrode surfaces during CV experiments.

3.6.4 Oxidative Electrolysis of Retinyl Palmitate (4)

Retinyl palmitate (**4**) displays a chemically irreversible oxidation process with a relatively small peak current compared to the rest of the compounds tested (except for retinol). However, unlike retinol the small peak current for (**4**) is probably partially a result of it having a much lower diffusion coefficient due to it being larger than the other compounds.

Figure 3.48: (a) CV of 1×10^{-3} M retinyl palmitate (**4**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of 0.4 electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinyl palmitate at $+0.54 \text{ V vs. Fc/Fc}^+$.

Exhaustive oxidative electrolysis of (**4**) involved the transfer of $0.4e^-$ per molecule (Figure 3.48). The low number of electrons transferred during the electrolysis suggest that the initially formed oxidized product is able to react with the starting material, thereby removing some of the starting material from the solution. CVs conducted after the electrolysis using a 1 mm GC electrode do not show any evidence of a new product which could be reduced or oxidised within the potential range scan.

Diagram 3.6: Oxidative electrolysis of retinyl palmitate (**4**). (a) Initial colourless solution. (b) After some time into the oxidation process, colourless solution turn lilac. (c) Starting material totally exhausted and lilac solution turned brown. (d) Finally, upon reaching $0.4e^-$ oxidation, solution changes to greenish yellow.

Diagram 3.6 shows how compound (**4**) in solution changes from a colourless starting state to various colours along the oxidation electrolysis pathways. The multiple changes in colour suggest not a straightforward conversion from a neutral to oxidized species but possibly multiple intermediate steps before finally reaching the final product. This could be expected for compound which has long conjugated chain and is susceptible to structural changes as reaction proceeds.

3.6.5 Oxidative Electrolysis of Retinyl Acetate (**5**)

Oxidative electrolysis of retinyl acetate (**5**) was conducted by holding the potential at $+0.53\text{ V vs. Fc/Fc}^+$, which led to the transfer of $0.5e^-$ for the exhaustive oxidation of (**5**) in CH_3CN .

Figure 3.49: (a) CV of 1×10^{-3} M retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of 0.5 electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinyl acetate at $+0.53 \text{ V vs. Fc/Fc}^+$.

After $0.5e^-$ had been passed, the oxidative current decreased to a very low level suggesting the completion of the electrolysis (Figure 3.49). Considering the anodic peak heights of (**1**), (**2**) and (**6**) obtained during CV measurements, the count of electrons of (**5**) would be expected to be more than $0.5e^-$. The initially oxidized product could have reacted with the starting material that is still within the solution, hence resulting in a lower count of electrons as the starting material is depleted from further reaction. The CV performed, after the electrolysis was completed did not show any new product which could be oxidized or reduced within the potential range scanned. The oxidation involved multiple colour changes in the electrolysis cell from colourless, to violet then very dark green solution (Diagram 3.7).

Diagram 3.7: Oxidative electrolysis of retinyl acetate (**5**). (a) Initial colourless solution. (b) After some time into the oxidation process, colourless solution turns violet. (c) Finally, upon reaching $0.5e^-$ oxidation, solution changes to dark green.

Lowering the temperature that the electrolysis was conducted at to 253 K did not appear to help in stabilizing any of the intermediate species that might have been formed, as there are still no new peaks detected from the CV after the electrolysis process (Figure 3.50). Electrolysis at 253 K also resulted in the transfer of $0.5e^-$ per molecule and similar violet/dark blue and finally green colours were observed during the procedure (Diagram 3.8).

Figure 3.50: (a) CV of 1×10^{-3} M retinyl acetate (**5**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 253 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of 0.5 electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinyl acetate at $+0.53 \text{ V vs. Fc/Fc}^+$.

Diagram 3.8: Oxidative electrolysis of retinyl acetate (**5**) at 253 K. (a) Initial colourless solution. (b) After some time into the oxidation process, colourless solution turns violet. (c) The solution reaches a stage where a dark blue colour is visible only at low temperature. (d) Finally upon reaching $0.5e^-$ oxidation, solution changes to dirty green.

3.6.6 Oxidative Electrolysis of Retinal (6)

Retinal is oxidized at $\sim +0.60$ V vs. Fc/Fc^+ on a 1 mm GC WE and 1 mm Pt WE in CH_3CN in a chemically irreversible process using 0.2M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) electrolyte. Oxidative electrolysis of retinal indicated that the reaction involved the transfer of $4e^-$ (Figure 3.51) which is equivalent to the number of electrons that was reported earlier by Park¹⁷ who conducted the experiment using THF as the solvent.

Figure 3.51: (a) CV of 2×10^{-3} M retinal (6) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of 4 electrons per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the oxidative electrolysis of retinal at $+0.62$ V vs. Fc/Fc^+ .

Conducting the electrolysis at 295 ± 2 K allows all $4e^-$ to be transferred without obvious complications. The solution changes from an initial yellow colour to a deep purple colour by the end of the experiment (Diagram 3.9). There are no intermediate colours detected during the process to reach $4e^-$.

Diagram 3.9: Oxidative electrolysis of retinal (**6**). (a) Initial yellow solution. (b) After reaching $4e^-$ oxidation, solution changes to deep purple.

The electrolysis oxidation reaction was also conducted by stopping the applied voltage after the transfer of one electron, and then recording a cyclic voltammogram of the solution using a 1 mm GC WE. Again, the experiment was stopped when it reached the next electron count and a CV scan was conducted to examine the CV transformation of (**6**) (Figure 3.52). The electrolysis was stopped when the overall number of electrons transferred was $2e^-$. The small reduction peak that was detected during CV experiments at -0.50 V vs. Fc/Fc^+ corresponds to the reduction of an oxidized product formed during the electrolysis. The peak size of the oxidized product does not substantially increase in size as the electrolysis progresses, suggesting that it is associated with an intermediate species rather than the long-term oxidation product.

Electrolysis was also performed at 253 K to see if lowering of temperature altered the number of electrons that are transferred compared to at 295 ± 2 K. At low temperatures, the electrolysis process appears to remain at less than $2e^-$ and reaches a very low electrolysis current. However, the reduction peak for the oxidized product has a bigger current compared to the peak for the oxidized product obtained at higher temperatures. Therefore, the low temperature may have helped to stabilize the oxidised product which might in turn react with the initial compound, so that the electrolysis is never able to reach $4e^-$ because the starting material has reacted with its oxidised product.

Figure 3.52: (a) CV of 2×10^{-3} M retinal (**6**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before, (....) after the transfer of one electron per molecule, (----) after the transfer of two electrons per molecule in a controlled potential electrolysis.

CVs scan was also conducted using 1 mm Pt WE (rather than a GC electrode) after the electrolysis in order to examine any difference there might be for a different electrode surface (Figure 3.53). A reduction peak was detected during CV experiments at $-0.53 \text{ V vs. Fc/Fc}^+$, which was not so obvious when the reaction was monitored in a GC electrode (Figure 3.51). The current of the reduction peak is bigger than the reduction peak obtained using the GC electrode and it corresponds to the reduction of a product of the initial oxidation. The product peak is slightly chemically reversible on the CV time scale at the end of the electrolysis.

Figure 3.53: CV of 2×10^{-3} M retinal (**6**) in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter Pt WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of four electrons per molecule in a controlled potential electrolysis cell.

The electrolysis oxidation reaction was again conducted by stopping the applied voltage after the transfer of one electron, and then recording a cyclic voltammogram of the solution using a 1 mm Pt WE. The same experimental procedure was repeated thrice by stopping when it reached the next electron count to examine the transformation of (**6**) (Figure 3.54a). The electrolysis was stopped when the overall number of electrons

transferred was $4e^-$. A large reduction peak was detected during the CV experiment at ~ -0.40 V vs. Fc/Fc^+ which corresponds to the reduction of the oxidized product formed during oxidative electrolysis. The anodic peak height decreases as the oxidative electrolysis proceeds. The peak size of the oxidized product substantially increases as the electrolysis progresses which is different compared to the result obtained using the GC electrode for monitoring the reaction (Figure 3.52).

Figure 3.54: CVs of 2×10^{-3} M retinal in CH_3CN containing 0.2 M Bu_4NPF_6 at (a) 295 ± 2 K and (b) 253 K at a 1 mm diameter Pt WE at a scan rate of 0.1 V s^{-1} ; (solid black line) before, (—) after the transfer of one electron per molecule, (....) after the transfer of two electrons per molecule, (----) after the transfer of three electrons per molecule, (-.-.-) after the transfer of four electrons per molecule in a controlled potential electrolysis cell where current/coulometry versus time data was obtained during the oxidative electrolysis of retinal at $\sim +0.63$ V vs. Fc/Fc^+ .

3.7 Summary Table of the number of Electrons for Vitamin A at Various Experimental Conditions

The following table shows a summary of the number of electrons involved in the oxidation and reduction processes for both carotenoid and retinoids at various temperatures on GC WE.

Compound	Reduction		Oxidation	
	Potential /V vs. Fc/Fc ⁺	No. of electron/s	Potential /V vs. Fc/Fc ⁺	No. of electron/s
(1) β -carotene	-2.14	2e ⁻	+0.38	2e ⁻
(2) Retinoic Acid	-1.90	1e ⁻	+0.61	2e ⁻
(3) Retinol	—	—	—	—
(4) Retinyl Palmitate	-2.24	2e ⁻	+0.54	0.4e ⁻
(5) Retinyl Acetate	-2.22	2e ⁻	+0.53	0.5e ⁻
(6) Retinal	-1.84	1e ⁻	+0.62	4e ⁻

Table 3.2: Number of electrons transferred and potential applied during oxidative and reductive controlled potential bulk electrolysis of compound (2) – (6) in CH₃CN and (1) in CH₂Cl₂.

3.8 Conclusion

A series of voltammetric experiments have shown similarities and differences in the electrochemical behavior of the different forms of vitamin A. Differences in the voltammetric responses were also detected when experiments were conducted at GC and Pt electrodes, in CH₃CN and CH₂Cl₂ solvents and at variable temperatures between 253 – 295 K.

The results indicated that retinal is the only vitamer which displays a chemically reversible reductive process when electrochemical experiments were conducted in CH₃CN using GC as the working electrode. Although the other compounds are all able to be reduced, they display chemically irreversible voltammetric processes up to the maximum scan rate measured of 10 V s⁻¹. The number of electrons transferred during each oxidation and reduction process for each vitamer has been compiled in Table 3.2

β -Carotene (1) is the only vitamer that shows a chemically reversible oxidation process. The oxidation occurs via two electrons and at very low temperatures (253 K) the dication partially survives during controlled potential electrolysis experiments to be detectable in the bulk solution.

The voltammetric results indicate that it is difficult to study these vitamers using a Pt working electrode because most of the compounds do not give a clear response within

the potential window available for the electrode. For example, when studying β -carotene, the reduction process on a Pt electrode displays overlapping of peaks of the analyte with the background. On Pt, retinoic acid does not display its second reduction process which is otherwise visible on a GC electrode.

Retinyl palmitate (**4**) and retinyl acetate (**5**) show interesting similarities in their voltammetric behavior. The structure of retinyl palmitate differs from retinyl acetate by a long saturated chain. The voltammetric behavior of both compounds is very similar except for retinyl acetate always registering a bigger peak current than retinyl palmitate. The number of electrons involved in the reduction process for both compound is two and the colour of the product after the bulk electrolysis are the same. It is possible that both species undergo very similar reductive reaction pathways. The oxidation of both compound do not give a full count of electrons (0.4 – 0.5) which again suggests a possible similar oxidative reaction pathway.

Retinol (**3**), which has a much shorter chain length than retinyl palmitate and differs from retinal by the terminating group, shows much smaller oxidative and reductive current responses from all of the other compounds during CV measurements. It is possible that it is the most sensitive to the external environment and that causes it to lose its electroactivity and hence only display very small oxidative and reductive peak currents.

3.9 References

1. Webster, R. D. *Chem. Rec.* **2012**, *12*, 188-200.
2. De Abreu, F. C.; Ferraz, P. A. L.; Goulart, M. O. F. *J Braz. Chem. Soc.* **2002**, *13*, 19-35.
3. Mernitz, H.; Wang, X-D. In *Vitamin A New Research*; Loessing, I. T., Eds.; Nova Biomedical Books: New York, 2007, 39-57.
4. Mathews, C. K.; Van Holde, K. E.; Ahern, K. G. *Biochemistry*, 3rd Ed.; Addison Wesley Longman, Inc.: San Francisco, 2000, 696-697.
5. Nagao, A. *J. Nutr.* **2004**, *134*, 237S-240S.
6. Willet, W. C.; Stampfer, M. J.; Underwood, B. A.; Taylor, J. O.; Hennekens, C. H. *Am. J. Clin. Nutr.* **1983**, *38*, 559-566.
7. Constantino, J. P.; Kuller, L. H.; Begg, L.; Redmond, C. K.; Bates, M. W. *Am. J. Clin. Nutr.* **1988**, *48*, 1277-1283.
8. Johnson, E. J.; Suter, P. M.; Sahyoun, N.; Ribaya-Mercado, J. D.; Russell, R. M. *Am. J. Clin. Nutr.* **1995**, *62*, 598-603.
9. Dryhurst, G. *Electrochemistry of Biological Molecules*, Academic Press: New York, 1977; Chapter 1, pp1-5.
10. Tan, Y. S.; Yue, Y.; Webster, R. D. *J. Phys. Chem. B* **2013**, *117*, 9371-9379.
11. Ziyatdinova, G.; Giniyatova, E.; Budnikov, H. *Electroanalysis* **2010**, *22*, 2708-2713.
12. Wring, S. A.; Hart, J. P.; Knight, D. W. *Analyst.* **1988**, *113*, 1785-1789.
13. Senoo, H. In *Vitamin A New Research*; Loessing, I. T., Eds.; Nova Biomedical Books: New York, 2007, 59-70.
14. Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R. *Pure Appl. Chem.* **1991**, *63*, 71-80.
15. Tan, Y. S.; Webster, R. D. *J. Phys. Chem. B* **2011**, *115*, 4244-4250.

16. Mairanovsky, V. G.; Engovatov, A. A.; Ioffe, N. T.; Samokhvalov, G. I. *J. Electroanal. Chem.* **1975**, *66*, 123-137.
17. Park, S-M. *J. Electrochem. Soc.* **1978**, *125*, 216-222.
18. Hapiot, P.; Simonet, J. In *Electroanalytical Chemistry: A Series of Advances (Volume 23)*, Bard, A. J.; Zoski, C. G., Eds.; CRC Press Taylor and Francis Group: Boca Raton, FL, 2010, 105-121.
19. Kildahl-Andersen, G.; Konovalova, T. A.; A. L.; Kispert, L. D.; Anthonsen, T.; Liaaen-Jensen, S. *Tetrahedron Lett.* **2007**, *48*, 8196-8199.
20. MacCrehan, W. A.; Schönberger, E. *Clin. Chem.* **1987**, *33*, 1585-1592.
21. Atuma, S. S.; Lindquist, J.; Lundström, K. *Analyst* **1974**, *99*, 683-689.
22. Atuma, S. S.; Lindquist, J.; Lundström, K. *Analyst* **1975**, *100*, 827-834.
23. Collins, C. M.; Leventis, N.; Sotiriou-Leventis, C. *Electrochim. Acta.* **2001**, *47*, 567-576.
24. Jeevarajan, J. A.; Jeevarajan, A. S.; Kispert, L. D. *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 1757-1765.
25. Liu, D. Z.; Gao, Y. L.; Kispert, L. D. *J. Electroanal. Chem.* **2000**, *488*, 140-150.
26. Jeevarajan, J. A.; Kispert, L. D. *J. Electroanal. Chem.* **1996**, *411*, 57-66.
27. Jeevarajan, A. S.; Khaled, M.; Kispert, L. D. *J. Phys. Chem.* **1994**, *98*, 7777-7781.
28. Jeevarajan, A. S.; Khaled, M.; Kispert, L. D. *Chem. Phys. Lett.* **1994**, *225*, 340-345.
29. Wang, L. -H. *Anal. Chim. Acta.* **2000**, *415*, 193-200.
30. Kissinger, P. T.; Heineman, W. R. *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Ed.; Marcel Dekker, Inc.: New York, 1996, 55-60.
31. He, Z.; Kispert, L. D. *J. Phys. Chem. B* **1999**, *103*, 10524-10531.
32. Takahashi, R. *Rev. Polarogr.* **1961**, *9*, 247-248.
33. Takahashi, R.; Tachi, I. *Agric. Biol. Chem.* **1962**, *26*, 771-776.

34. Kuta, E.; Yu, M. *Lipids* **1967**, 2, 411-418.
35. Kuta, E. *Science*. **1964**, 144, 1130-1131.
36. Valentin Pfund, B.; Bond, A. M.; Hughes, T. C. *Analyst* **1992**, 117, 857-861.

Chapter 4

Competing Hydrogen-Bonding, Decomposition and Reversible Dimerization Mechanisms During the One- and Two-Electron Electrochemical Reduction of Retinal (Vitamin A)

4.1 Introduction

Retinal is the only vitamer which displayed a chemically reversible reductive process when electrochemical experiments were conducted in CH_3CN using a GC working electrode, as described in Chapter 3. The detailed study of the reduction mechanism of retinal (**6**) is described in this Chapter.

Retinal (Scheme 4.1) is a form of vitamin A found in plants and is thought to be the product from the oxidative cleavage of β -carotene.¹⁻³ Being a liposoluble vitamin, after ingestion it is stored in the fat cells in the body, where it can also undergo various chemical conversion processes.⁴⁻⁷ In physiological reactions, retinal is recognized as being used as a visual pigment in the eye in order to enhance night vision.⁸ Retinal is also thought to be beneficial as an antioxidant,⁵ as well as having preventive effects on various diseases and cancers.⁶⁻¹⁰ As it exists in epithelial cells in the skin, it helps to prevent skin diseases by acting as a first line of defense. Since the transformations between the different forms of vitamin A can involve oxidation or reduction mechanisms inside lipophilic membranes, it is interesting to examine the voltammetric properties of the compound in a low-water environment.

Scheme 4.1: Structure of all-trans retinal

Several detailed studies have been performed on electrochemical reaction mechanisms related to carotenoid compounds having similar molecular structures as retinal.¹¹⁻¹³ Even though retinal is a common and beneficial form of vitamin A, little is known of the exact electrochemical reaction mechanism it undergoes. It has been proposed that retinal undergoes an initial one-electron reduction to generate an anion radical.¹⁴⁻¹⁵ The homogeneous reactions following the generation of the anion radical have led to some uncertainty as to whether a dimerization process takes place or a hydrolysis reaction with trace water in the solvent (or both processes). Previous studies have tried to decipher whether a protonation reaction or dimerization reaction takes place by using various methods such as electrochemical kinetic studies,¹³ theoretical calculations,¹⁴ and spectroscopic experiments.^{14,15} Some studies have also proposed a disproportionation step where the doubly reduced retinal (a dianion) reacts with the starting material to form two molecules of the anion radical.¹⁴ The actual site of the molecule undergoing reduction is still unclear. It could possibly occur within the polyene chain or at the carbonyl group.¹⁶ Electrochemical reduction experiments have been performed on activated olefins and it has been shown that they undergo dimerization through their associated anion radicals.¹⁷⁻²⁰ In alkaline ethanol solutions, benzaldehyde undergoes reductive dimerization through its radical anion.^{21,22} It has been proposed that reduced retinal undergoes dimerization when proton donors are present, similar to malonate esters.²³⁻²⁴

In this Chapter, we have used variable scan rate cyclic voltammetry combined with digital simulation modelling of the data to better understand the reduction mechanism of retinal. The focus was on determining whether the reduced species underwent hydrogen-bonding interactions with water, hydrolysis (protonation reactions) or whether the simulations supported a dimerization reaction of the anion radicals.

4.2 Results and Discussion

4.2.1 Cyclic Voltammetry of Retinal in Acetonitrile with Low Water Concentrations

Acetonitrile (CH_3CN) was used in this study because it is less hygroscopic than other aprotic solvents suitable for electrochemistry such as dimethyl sulfoxide and dimethylformamide, enabling the initial water content to be more easily maintained at a relatively low level.³¹ While chlorinated solvents such as dichloromethane and 1,2-dichloroethane are less hygroscopic than CH_3CN , they have a limited reductive potential range that makes it difficult to detect all of the voltammetric processes of retinal.

Figure 4.1: (a) CV of 2×10^{-2} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 50 (\pm 10)$ mM) containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC electrode at a scan rate of 0.1 V s^{-1} ; (solid black line) before and (dotted red line) after the transfer of one electron per molecule in a controlled potential electrolysis cell. (b) Current/coulometry versus time data obtained during the reductive electrolysis of retinal at $-1.90 \text{ V vs. Fc/Fc}^+$.

Voltammograms of retinal in CH_3CN containing 0.2 M Bu_4NPF_6 at room temperature (295 ± 2 K) show two reduction processes at negative potentials (solid black line in Figure 4.1a, peaks I_{red} and II_{red}). Bulk controlled potential electrolysis (with

coulometry) was conducted to find the number of electrons transferred in the first reduction process (Figure 4.1b). The coulometry experiments indicated that the first reduction peak was a one-electron process, which resulted in the formation of the radical anion $R^{\bullet-}$.

At a scan rate of 0.1 V s^{-1} , both the first and second reduction processes display much smaller reverse oxidative peak currents (i_p^{ox}) compared to their forward reductive peak currents (i_p^{red}), where ($i_p^{\text{ox}}/i_p^{\text{red}} \ll 1$), which is due to chemical instability of the reduced compounds (Figure 4.2). An oxidation peak (III_{ox}) appears at a potential of approximately -1.0 V vs. Fc/Fc^+ only if the scan is first applied in the negative potential direction past the first one-electron reduction process and is thus associated with the oxidation of a secondary product of the reduction. Multiple scans showed no reverse (reduction) peak for process III_{ox} indicating that the generated product is itself short-lived when it is oxidized. It is possible that other secondary products have also been formed after the reduction processes and are not redox active or do not fall within the potential window of CH_3CN (using a GC electrode).

When the scan direction is reversed after the second reduction process at approximately -2.20 V vs. Fc/Fc^+ , two small oxidation processes are detected (I_{ox} and II_{ox}) which are the reverse processes of I_{red} and II_{red} , respectively. The first reduction process (I_{red}) is due to the formation of the radical anion, and the second reduction process (II_{red}) is the further one-electron reduction of the radical anion to form the dianion (R^{2-}). At slow scan rates ($< 10 \text{ V s}^{-1}$), the i_p^{red} value for the second process is smaller than the i_p^{red} value for the first process because the radical anion is undergoing other homogeneous reactions before it has time to be reduced to the dianion. Halting the forward potential scan just after the first electron transfer process (Figure 4.2) resulted in an increase in the size of the i_p^{ox} value for process I_{ox} because there were more radical anions at the electrode surface able to undergo oxidation back to the starting material. However, the

subsequent oxidation peak at approximately -1.00 V vs. Fc/Fc^+ becomes much smaller, suggesting that the species responsible for this process are formed in a higher amount after the second reduction step. Another possible explanation is that process III_{ox} is associated with the oxidation of multiple species formed from homogeneous reactions of $\text{R}^{\bullet-}$ and R^{2-} , that are coincidentally oxidized at similar potentials. Experiments were also conducted by scanning values more negative than the second reduction peak potential; however, there was no obvious reduction process following the second reduction.

Figure 4.2: CV of 2×10^{-3} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 50 (\pm 10)$ mM) containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at a scan rate of 0.1 V s^{-1} .

Background voltammograms of just the solvent and electrolyte were performed at all scan rates to reduce contributions from the charging current and minor solvent impurities detected with the use of the 1 mm diameter GC working electrode. All CV data are presented minus their backgrounds to more clearly show the retinal peaks under the reaction conditions stated. CV data of 2×10^{-3} M retinal in CH_3CN containing 0.2 M Bu_4NPF_6 recorded at 1 mm diameter GC working electrode at variable scan rates show that as the scan rate increases, the first reduction peak becomes more chemically reversible (Figure 4.3). The oxidation peak (III_{ox}) at approximately -1.0 V vs. Fc/Fc^+ displays a large difference in peak current depending on whether the scan direction is switched immediately after the first reduction or the second reduction process. It appears that the species responsible for process III_{ox} are produced from both the first and second one-electron reduction steps, but a larger proportion of the product comes after the generation of the dianion.

Figure 4.3: CV of 2×10^{-3} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 50 (\pm 10)$ mM) containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at variable scan rates (ν) of 0.1, 0.5 and 1.0 V s^{-1} . The current axis has been scaled by multiplying by $\nu^{-0.5}$.

Variable scan rates experiments were also conducted at several different temperatures; 295 K, 283 K, 273 K, 263 K, 253 K and 243 K (Figure 4.4). It was found that the chemical reversibility of the first and second electron transfers steps did not change substantially with changing temperature, indicating that the lifetime of the dianion did not noticeably improve at low temperatures. Even at a scan rate of 20 V s^{-1} , the reverse oxidation peak (II_{ox}) remains very small. The peak currents of the reduction and oxidation processes decreased as the temperature was lowered because of slower diffusion rates of the species involved. Some interesting changes with temperature were observed for process III_{ox} .

Figure 4.4: CVs of 2×10^{-3} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 50 - 100$ mM) containing 0.2 M Bu_4NPF_6 at variable temperatures recorded at a 1 mm diameter GC working electrode at scan rate of 0.5 V s^{-1} .

As Figure 4.4a shows, the oxidation process III_{ox} which occurs at approximately $-1.00 \text{ V vs. Fc/Fc}^+$ at 295K, shifts more positively to approximately $-0.90 \text{ V vs. Fc/Fc}^+$ at 243K. The peak also changes from a sharp peak at higher temperatures to a flattened peak at lower temperatures, possibly because of the separation into two electron transfer processes. Examination of Figure 4.4b, in which the potential was switched just after the first electron transfer step, reveals that the small oxidation peak at approximately $-1.00 \text{ V vs. Fc/Fc}^+$ also shifted more positively with decreasing temperatures. The III_{ox} process in the right column is always at a potential that is slightly more positive potential than that

of the III_{ox} process in the left column where the switching potential is more negative. This indicates that it is possible that different secondary products (but with similar oxidation potentials) were generated depending on whether the scan direction is switched just after the first or second electron transfer reduction process. Therefore, different reaction pathways exist for the radical anion and dianion generated after the initial heterogeneous electron transfer reduction steps.

Experiments with 1×10^{-3} M, 2×10^{-3} M and 5×10^{-3} M retinal were performed in order to assess concentration effects on the voltammetric reduction mechanism (Figure 4.5). As the concentration increased, the second reduction peak decreased in size relative to the first reduction process, showing that with increasing concentration of retinal, fewer radical anions are able to undergo further reduction to the dianion. Furthermore, as the concentration of retinal increased, the chemical reversibility of the first reduction process appeared to decrease. These results provide evidence of a dimerization process after the formation of the radical anion (eq 4.1).

The change in peak current ratios can also be seen clearly in Figure 4.1 in which a very high 20 mM concentration of retinal shows a second reduction process (II_{red}) that is much smaller than the first process (I_{red}) at a scan rate of 0.1 V s^{-1} . When the radical anion is generated after the first reduction step, two molecules can react together and dimerize, resulting in fewer radical anions being reduced in the second reduction process. With a greater concentration of starting material, it is more likely that the radical anions can react; hence, more dimerized product will be formed. This can also be voltammetrically observed by looking at Figure 4.5b, where a greater concentration of retinal of (5×10^{-3} M) gives a less chemically reversible first reduction peak compared to that of lower concentrations, showing that more of the radical anions had dimerized. It can be observed in the voltammograms in Figure 4.5b that the oxidation peak at

approximately -1.00 V vs. Fc/Fc^+ (III_{ox}) appears relatively larger (compared to the oxidation peak (I_{ox})) at high concentrations compared to low concentration. This suggests that process III_{ox} , which is evident when the scan is reversed just after the first one-electron reduction, can be assigned to the oxidation of the newly formed dimerized species.

Figure 4.5: CV of 1×10^{-3} M, 2×10^{-3} , 5×10^{-3} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 60 (\pm 10)$ mM) containing 0.2 M Bu_4NPF_6 at 263K obtained at a 1 mm diameter GC working electrode at scan rate of 0.5 V s^{-1} with different switching potentials.

The peak height registered at lower concentration is smaller because less dimerized product is formed. This in turn gives a more chemically reversible peak for the first reduction process at lower concentrations. There are several reports of the radical anions of aromatic compounds produced by electrochemical methods undergoing reversible dimerization reactions.³²⁻³⁸ The dimers of the radical anions have been reported to be more difficult to oxidize than the radical anions, which results in cyclic voltammograms showing an oxidation process a few hundred mV more positive than the oxidation of the radical anion (i.e., consistent with process III_{ox}).

Synthetic scale controlled potential electrolysis experiments were performed by electrolyzing 140 mg of retinal. Thin-layer chromatography analysis of the reaction mixture at the completion of the electrolysis showed a number of products that were not easily separated and purified by preparative column chromatography. Nevertheless, ^{13}C NMR spectroscopy performed on the entire reaction mixture showed no carbonyl carbon resonance, indicating that the aldehyde group had undergone reduction. Similarly, Fourier transform infrared analysis of the reaction mixture did not lead to the detection of a carbonyl stretching band. Therefore, it is possible that the dimerization occurs through the carbonyl group to form a pinacol, as has been previously proposed.²³ However, we were not able to obtain spectroscopic evidence for the existence of the dimer on the synthetic time scale; thus, it is equally probable that the dimer exists only as an intermediate in solution, which is often the case in reversible dimerization reactions.³²⁻³⁸ Furthermore, because of the relatively high reduction potential, it is unlikely that dianionic dimers would survive as intermediates under the conditions used for liquid chromatography-mass spectrometry or gas chromatography-mass spectrometry experiments.

The experiments conducted with varying temperature, scan rate and concentration help identify the likely reduction mechanism that retinal undergoes, which is consistent with a radical dimerization mechanism (radical anion coupling) if the potential is extended only to the first reduction process. However, the reverse oxidation process (III_{ox}) that is observed when the potential is extended past the second one-electron transfer to form the dianion may not just be associated with dimer formation.

4.2.2 Cyclic Voltammetry of Retinal in Acetonitrile with Variable Water Concentrations

Previous studies have indicated that water plays a role in the reduction mechanism of retinal in organic solvents;^{23,24} thus, it is important to quantitatively determine exactly how water affects the electrochemical behavior of the starting material and the

electrochemically generated species.³⁹ Water is an ever-present impurity in organic solvents and normally exists in a substantially higher concentration than the analyte, unless scrupulous care is taken to remove it.³¹ Electrochemical reactions involving the accurate addition of water are difficult to perform because of natural contamination from the atmosphere into the electrochemical cell over time. Although an initial water content of 20 mM can be achieved in the electrochemical cell at room temperature, the value quickly increases, especially at lower temperatures.

With a water content of 20 mM, it was observed that peaks I_{ox} and III_{ox} were a similar size at a scan rate of 0.1 V s^{-1} (Figure 4.6). This was not the same as the voltammogram obtained with a higher water content (Figure 4.5) in which III_{ox} appears larger than I_{ox} at the same scan rate and retinal concentration. This result indicates that the species responsible for III_{ox} is both a dimeric compound (formed by dimerization of the radical anion) and a reaction product that is favored in the presence of water.

When water was progressively added to the solvent and voltammograms were conducted over a range sufficiently negative to form only the anion radical (Figure 4.6b), oxidation process III_{ox} became relatively larger compared to oxidation process I_{ox} , suggesting that oxidation process III_{ox} is partly associated with a reaction product of $R^{\bullet-}$ (in addition to a dimeric species). Similarly, when the forward potential scan was extended so as to form the dianion (Figure 4.6a), process III_{ox} became much larger as more water was added to the solvent (up to 1 M), suggesting that the dianion undergoes a decomposition reaction and that this occurs to a greater extent than for the anion radical (by a comparison of Figure 4.6a and b). At very high water concentrations ($> 0.5 \text{ M}$), process III_{ox} substantially decreases in size and takes on a more complicated shape, possibly because of competing hydrolysis reactions making new compounds that are oxidized at different potentials.

Figure 4.6: CV of 5×10^{-3} M retinal in CH_3CN containing 0.2 M Bu_4NPF_6 at 295 ± 2 K at a 1 mm diameter GC working electrode at scan rate of 0.1 V s^{-1} with a varying amount of water.

It was also noticed that the second reduction peak changes in shape as more water is added to the solution, becoming progressively flatter. The peak potential shifts to more positive potentials as water is added, making it easier to reduce the starting material and radical anion. The shift in potential can be attributed to a hydrogen-bonding mechanism that is often observed during the reduction of quinones, where the hydrogen-bonding

facilitates the reduction processes.⁴⁰⁻⁴² Although it could be argued that process III_{ox} is actually associated simply with the oxidation of a hydrolyzed product of the anion radical and dianion (or a product favored in the presence of water), this is not consistent with the fact that process III_{ox} becomes larger (relative to process I_{ox}) when the concentration of retinal is increased (Figure 4.5). As the concentration of retinal increases, there are actually fewer water molecules per molecule of retinal; thus, it would be expected that a reaction product that is favored in the presence of water would result in process III_{ox} becoming relatively smaller compared to process I_{ox}, which is the opposite of what is observed. Therefore, it is proposed that process III_{ox} is associated with oxidation of both a dimeric compound (R_2^{2-}) that forms via dimerization of $R^{\bullet-}$ as well as a decomposition product X formed from R^{2-} that is favored in the presence of H₂O.

4.2.3 Digital Simulation of CV Measurements on Retinal with Variable Water Concentrations and Temperature

Digital simulation studies were performed to determine the most appropriate mechanism to account for the voltammetric behavior of retinal. The simulations were performed by a trial and error process that involved systematically expanding the steps in the electrochemical mechanism using one set of parameters over all scan rates (for each individual temperature) until the simulated voltammograms matched the experimental voltammograms. Initially, the diffusion coefficient (D) of retinal was determined from the reductive peak current of the first process, while the D -values for the other compounds (anions and dimers) were lowered by up to 50% to take into account the stronger electrolyte interactions and larger size slowing down their movement. The fitting procedure was performed by eye rather than by an automated defined error minimization process. The match between representative simulations and experimental data is shown in Figure 4.7.

Scheme 4.2: Electrochemical reduction mechanism of retinal in CH_3CN deduced by digital simulation of cyclic voltammetry data over a range of scan rates, temperatures, retinal concentrations and water concentrations. The chemical equilibrium and kinetic values associated with the heterogeneous electron transfer and homogeneous chemical reactions are given in Table 4.1.

Scheme 4.2 shows the reaction mechanism of the electrochemical reduction of retinal that was deduced by digital simulation of the series of cyclic voltammetry scans conducted at variable scan rates, temperatures, amounts of water and retinal concentrations. The CV scans were performed over two potential ranges: one involved the initial one-electron reduction of retinal, and the other was extended to a sufficiently negative potential to further reduce the radical anion to the dianion. Therefore, the

mechanism shown in Scheme 4.2 is dependent on the applied potential with partially different pathways occurring for the one- and two-electron reductions.

At slow scan rates, the one-electron reduction of retinal produces the radical anion ($R^{\bullet-}$) which quickly dimerizes to form R_2^{2-} . This dimerization process also occurs after a hydrogen-bonding step in which $R^{\bullet-}$ interacts with water to form $R^{\bullet-}(H_2O)$, which then dimerizes to form $R_2^{2-}(H_2O)_2$. In addition to combining with water and undergoing dimerization, the radical anion can be further reduced at more negative potentials to form a dianion R^{2-} . The fact that the one-electron reduction of the anion radical to form the dianion is not chemically reversible (at scan rates $< 20 \text{ V s}^{-1}$) indicates a fast homogeneous reaction step following the heterogeneous electron transfer,⁴³ resulting in the generation of a new species that can be oxidized at more positive potentials (III_{ox}). Although process III_{ox} becomes larger when water is added to solutions, the simulations did not support a straight hydrolysis reaction. Instead, a better match of the simulations to the experimental data could be obtained for a decomposition step to form a currently unidentified product X (Scheme 4.2). Process III_{ox} can also be detected on the reverse scan when the forward scanning potential is switched just after the first electron transfer step, but it is coincidentally associated with oxidation of the dimer dianion.

It was not possible to match the experimental with the simulated data exactly with just one unique set of data for all conditions because of the large number of steps involved. The data given in Table 4.1 show the best average fits for all of the data used at several temperatures for the reactions in Scheme 4.2. The solution resistance and diffusion coefficient values differed for each of the temperatures used. The general trend was that the resistance increased and the diffusion coefficient values decreased as the temperature was lowered.

The dimerization reaction was modeled on both a radical coupling mechanism (EC_{dim}) and an ECE_{dim} mechanism. The ECE_{dim} mechanism involves the radical anion

first reacting with a molecule of the starting material to form $R_2^{\bullet-}$, which then undergoes a further one-electron reduction to form R_2^{2-} . A closer match of the simulations to the experimental data was obtained for the EC_{dim} mechanism, which was modeled to involve both the H-bonded and non H-bonded radical anions. The simulations indicate that the equilibrium constants for the dimerization reactions (eqs 5 and 6 in Scheme 4.2) increase as the temperature decreases, which is similar to the results obtained from other electrochemical studies on reversible dimerization reactions indicating that the dimerization is favored at lower temperatures.³²⁻³⁸

Table 4.1: Equilibrium and rate constants obtained by digital simulation of CV data for the reaction mechanism given in Scheme 4.2.

Temperature / K	Kinetic parameters	Eq 1	Eq 2	Eq 3	Eq 4	Eq 5	Eq 6
295 ± 2 (DRY)	K_{eq}	5×10^4	1×10^7	1×10^3	1×10^4	5×10^4	3×10^4
	k_f	2×10^1	3×10^2	1×10^2	1×10^2	5×10^3	5×10^2
	k_b	4×10^{-4}	3×10^{-5}	1×10^{-1}	1×10^{-2}	1×10^{-1}	2×10^{-2}
295 ± 2	K_{eq}	2×10^4	2×10^5	3×10^3	8×10^3	5×10^4	4×10^4
	k_f	2×10^0	1×10^2	3×10^2	5×10^2	5×10^3	8×10^2
	k_b	1×10^{-4}	5×10^{-4}	1×10^{-1}	6×10^{-2}	1×10^{-1}	2×10^{-2}
273	K_{eq}	1×10^4	2×10^5	5×10^3	5×10^3	1×10^5	5×10^5
	k_f	2×10^0	1×10^2	3×10^2	5×10^2	5×10^3	1×10^3
	k_b	2×10^{-4}	5×10^{-4}	6×10^{-2}	1×10^{-1}	5×10^{-2}	2×10^{-3}
253	K_{eq}	5×10^3	2×10^5	8×10^3	1×10^3	2×10^5	1×10^6
	k_f	2×10^0	1×10^2	5×10^2	1×10^2	2×10^3	6×10^2
	k_b	4×10^{-4}	5×10^{-4}	6×10^{-2}	1×10^{-1}	1×10^{-2}	6×10^{-4}

CV data recorded in CH₃CN with 0.2 M n-Bu₄NPF₆ at a 1 mm diameter GC electrode at scan rates of 0.1 – 20 V s⁻¹ and at water concentrations between 0.020 and 0.1 M, where 0.020 was used for the dry conditions and 0.05 – 0.1 M for non-controlled conditions. Heterogeneous rate constants were estimated to be 0.5 cm s⁻¹. Diffusion coefficient values for retinal were 1.25×10^{-5} cm² s⁻¹ at 295 K, 9.00×10^{-6} cm² s⁻¹ at 273 K, and 6.00×10^{-6} cm² s⁻¹ at 253 K. The diffusion coefficient value for water was set at 1.00×10^{-5} cm² s⁻¹. Homogeneous rate constants for the forward (k_f) and backward (k_b) reactions have units of s⁻¹ and L mol⁻¹ s⁻¹ for the first and second-order reactions, respectively.

Although Figure 4.7 shows that the simulations could be made to closely fit the experimental data, the large number of parameters used in the simulations means that the values given in Table 4.1 are not necessarily a unique set. A further complication is that many of the parameters are interrelated in such a way that varying one necessitates

altering the others to maintain the close fit. Nevertheless, it was found that in all instances the degree of variation (and the estimated error) was substantially less than 1 order of magnitude, which is a reasonable degree of precision considering the large number of steps in the mechanism.

A set of "dry" experimental results were also simulated to fit the measured water content of 20 mM at room temperature while normal non-water controlled conditions used a water concentration of 50 ($\pm$ 10) mM.

Figure 4.7: CV data of 5×10^{-3} M retinal in CH_3CN ($[\text{H}_2\text{O}] = 50 (\pm 10)$ mM) containing 0.2 M Bu_4NPF_6 at 295 ± 2 K obtained at a 1 mm diameter GC working electrode at variable scan rates. Current data were scaled by multiplying by $\nu^{-0.5}$. The dotted red lines represent the simulated voltammograms according to the mechanism in Scheme 4.2 and parameters used in Table 4.1, while the solid black lines are the experimental data.

The simulations indicated that the potential of the first reduction process ($E^\circ_{(1)}$) occurred at $-1.75 (\pm 0.04)$ V vs. Fc/Fc^+ while the second reduction process ($E^\circ_{(2)}$) occurred

at $-2.15 (\pm 0.04)$ V vs. Fc/Fc^+ . The reduction potential ($E^\circ_{(3)}$) of the strongly hydrogen-bonded (with water) radical anion occurred at a slightly more positive potential of $-2.05 (\pm 0.02)$ V vs. Fc/Fc^+ . Process III_{ox} consists of multiple species undergoing oxidation ($E^\circ_{(4,5)}$) at slightly varying potentials ranging from -0.83 V vs. Fc/Fc^+ to -1.08 V vs. Fc/Fc^+ . Oxidation of the dimer dianion that is formed from two molecules of the anion radical takes place at a potential that is slightly more positive than that of the oxidation of product X that is formed via a decomposition reaction of the dianion. The assignment of peak III_{ox} as consisting of at least two processes was deduced by comparing the voltammograms obtained with different switching potentials. The oxidation process (III_{ox}) that was detected when the scan direction was reversed just after I_{red} occurred at a potential that was more positive than that of the oxidation process detected when the scan direction was reversed after II_{red} . Furthermore, it was found that when water was progressively introduced into the solution, the reduction peak potentials shifted to more positive potentials, which is likely due to hydrogen-bonding interactions.³⁹⁻⁴²

4.3 Conclusion

The results of this study indicate that retinal undergoes a series of homogeneous reactions following heterogeneous electron transfer that critically depend on retinal concentration, temperature, and water content of the acetonitrile solutions. At low water concentrations, low temperature, and high retinal concentrations, dimerization of the anion radical is the favored reaction mechanism. As the water content increases, hydrolysis reactions (including hydrogen-bonding) and decomposition of the dianion of retinal dominate. The electrochemical mechanism was particularly difficult to study because both major products of the reduction (dimer dianions and decomposition products) were themselves oxidized at very similar potentials, comprising oxidation process III_{ox} .

The experimental apparatus used for electrochemical experiments makes it difficult to completely exclude water from electrochemical cells. Furthermore, the requirement that voltammetric experiments be performed at relatively low analyte concentrations (< 10 mM) to avoid deleterious effects in modeling the data (such as IR drop and migration), mean that in most circumstances there will be more water than substrate. Therefore, the full interpretation of the voltammetric behavior of retinal requires knowledge of the water content of the solution. Whether these reactions occur biologically remains to be determined, but it is interesting that even relatively small amounts of water can substantially alter the reduction mechanism. Furthermore, it is also likely that other hydrogen-bonding donors such as amines, which are present in lipophilic membrane environments, will also affect the electrochemical behavior of retinal.

4.4 References

1. McMurry, J. E.; Fleming, M. P. *J. Am. Chem. Soc.* **1974**, 96, 4708–4709.
2. Nagao, A. *J. Nutr.* **2004**, 134, 237S–240S.
3. Ziyatdinova, G.; Giniyatova, E.; Budnikov, H. *Electroanalysis* **2010**, 22, 2708–2713.
4. Krinsky, N. I.; Yeum, K. -J. *Biochem. Biophys. Res. Commun.* **2003**, 305, 754–760.
5. Loessing, I. T. *Vitamin A New Research*, Nova Biomedical Books: New York, 2007, 39–57.
6. Ball, G. F. M. *Vitamins: their role in the human body*, 1st Ed.; Oxford, U.K.; Blackwell Pub. ; Ames, Iowa: Blackwell Professional Pub., 2004.
7. Webster, R. D. *Chem. Record.* **2012**, 12, 188–200.
8. Suarez-Fernandez, A. L.; Alarnes-Varela, G.; Costa-Garcia, A. *Electrochimica Acta* **1999**, 44, 4489–4498.
9. Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of Biochemistry*, W. H. Freeman: New York, 2000.
10. Murray, R. K.; Granner, D. K.; Mayes, P. A.; Rodwell, V. W. *Harper's Illustrated biochemistry*, 26th ed.; McGraw-Hill Medical: New York, 2003.
11. Mairanovsky, V. G.; Engovatov, A. A.; Ioffe, N. T.; Samokhvalov, G. I. *J. Electroanal. Chem.* **1975**, 66, 123–137.
12. Burton, G. W.; Ingold, K. U. *Science* **1984**, 224, 569–573.
13. Park, S. -M. *J. Electrochem. Soc.* **1978**, 125, 216–222.
14. Lang, C. M.; Harbour, J.; Guzzo, A. V. *J. Phys. Chem.* **1971**, 75, 2861–2866.
15. Powell, L. A.; Wightman, R. M. *J. Electroanal. Chem.* **1980**, 106, 377–390.
16. Nelson, A. *J. Electroanal. Chem.* **1992**, 335, 327–343.
17. Costentin, C.; Savéant, J. M. *J. Electroanal. Chem.* **2004**, 564, 99–113.

18. Sioda, R. E.; Terem, B.; Utley, J. H. P.; Weedon, B. C. L. *J. Chem. Soc. Perkin Trans. I* **1976**, 561–563.
19. Sopher, D. W.; Utley, J. H. P. *J. Chem. Soc. Chem. Commun.* **1979**, 1087–1088.
20. Lamy, E.; Nadjó, L.; Savéant, J. M. *J. Electroanal. Chem.* **1974**, 50, 141–145.
21. Savéant, J. M.; Tessier, D. *J. Phys. Chem.* **1978**, 82, 1723–1727.
22. Andrieux, C. P.; Grzeszczuk, M.; Savéant, J. M. *J. Am. Chem. Soc.* **1991**, 113, 8811–8817.
23. Powell, L. A.; Wightman, R. M. *J. Am. Chem. Soc.* **1979**, 101, 4412–4413.
24. Powell, L. A.; Wightman, R. M. *J. Electroanal. Chem.* **1981**, 117, 321–333.
25. Rudolph, M. *J. Electroanal. Chem.* **2003**, 543, 23–39.
26. Rudolph, M. *J. Electroanal. Chem.* **2003**, 558, 171–176.
27. Rudolph, M. *J. Electroanal. Chem.* **2004**, 571, 289–307.
28. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 619–632.
29. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 633–641.
30. Rudolph, M. *J. Comput. Chem.* **2005**, 26, 1193–1204.
31. Hui, Y.; Webster, R. D. *Anal. Chem.* **2011**, 83, 976–981.
32. Hammerich, O.; Parker, V. D. *Adv. Phys. Org. Chem.* **1984**, 20, 55–189.
33. Amatore, C.; Garreau, D.; Hammi, M.; Pinson, J.; Savéant, J. M. *J. Electroanal. Chem. Interfacial Electrochem.* **1985**, 184, 1–24.
34. Webster, R. D. *J. Chem. Soc. Perkin Trans. II* **1999**, 263–270.
35. Mazine, V.; Heinze, J. *J. Phys. Chem. A* **2004**, 108, 230–235.
36. Macías-Ruvalcaba, N. A.; Felton, G. A. N.; Evans, D. H. *J. Phys. Chem. C* **2009**, 113, 338–345.
37. Macías-Ruvalcaba, N. A.; Evans, D. H. *J. Phys. Chem. C* **2010**, 114, 1285–1292.
38. Haubner, K.; Tarábek, J.; Ziegs, F.; Lukeš, V.; Jaehne, E.; Dunsch, L. *J. Phys. Chem. A* **2010**, 114, 11545–11551.

39. Tan, Y. S.; Chen, S.; Hong, W. M.; Kan, J. M.; Kwek, S. H. E.; Lim, S. Y.; Lim, Z. H.; Tessensohn, M. E.; Zhang, Y.; Webster, R. D. *Phys. Chem. Chem. Phys.* **2011**, *13*, 12745–12754.
40. Gupta, N.; Linschitz, H. *J. Am. Chem. Soc.* **1997**, *119*, 6384–6391.
41. Quan, M.; Sanchez, D.; Wasylkiw, M. F.; Smith, D. K. *J. Am. Chem. Soc.* **2007**, *129*, 12847–12856.
42. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D. *J. Am. Chem. Soc.* **2009**, *131*, 1523–1534.
43. Bauer, P. -J.; Carl, P. *J. Am. Chem. Soc.* **1977**, *99*, 6850–6855.

Chapter 5

Electron-Transfer Reactions Between the Diamagnetic Cation of α -Tocopherol (Vitamin E) and β -Carotene (Provitamin A)

5.1 Introduction

α -Tocopherol (α -TOH), the most biologically active form of vitamin E, and β -carotene (β -Car) are produced by plants and when consumed by mammals reside inside the lipid bilayer membranes in living cells.¹ The widely accepted view is that α -TOH's major function in mammalian tissues is as an antioxidant, essentially a sacrificial compound that prevents cell membranes from turning rancid and decomposing.²⁻⁴ An alternative, although less accepted view, is that α -TOH has a specific role as a cellular signalling molecule, and its antioxidant properties are of secondary or little importance biologically.⁵⁻⁸ It is interesting to note that while α -TOH does unquestionably have antioxidant properties by virtue of its labile hydrogen atom on its hydroxyl group, many of the potential antioxidant benefits of consuming large doses of vitamin E are not realized in clinical trials.⁹ Therefore, the nonantioxidant functions of α -TOH are attracting increasing interest.¹⁰

β -Car has an important dual role in nature as a photoprotecting agent and a light-harvesting antenna.¹¹ β -Car's main function in humans is as a pro-vitamin, where it converts into vitamin A via an enzymatic process in the intestines, although it has been reported that it also displays antioxidant properties.¹² A pulse radiolysis study initially concluded that the oxidized form of α -TOH (α -TO $^{\bullet}$) could react with β -Car (plus a proton) to form the radical cation (β -Car $^{\bullet+}$), thereby regenerating α -TOH (eq 5.1).¹³ However, a following EPR study indicated that eq 5.1 was unlikely to occur.¹⁴ Therefore, a subsequent pulse radiolysis study investigated the interactions between the one-

electron-oxidized form of α -TOH (α -TOH $^{\bullet+}$) and β -Car, and it was suggested that β -Car can regenerate α -TOH from its one-electron-oxidized form (eq 5.2).¹⁵

The reaction in eq 5.2 is certainly electrochemically feasible based on the observation that α -TOH $^{\bullet+}$ is a powerful enough oxidant to oxidize β -Car. Nevertheless, electrochemical studies have demonstrated that α -TOH¹⁶⁻²⁹ and β -Car³⁰⁻³⁴ normally undergo two-electron oxidation at electrode surfaces in aprotic organic solvents such as CH₃CN and CH₂Cl₂. Therefore, in this Chapter, this study is directed at determining whether a homogeneous two electron reaction can occur between oxidized α -TOH and neutral β -Car.

The electrochemical oxidation mechanism for α -TOH in acetonitrile^{16,17,19-28} and dichloromethane^{17,20} is given in Scheme 5.1. α -TOH is initially oxidized by one electron to form the radical cation (α -TOH $^{\bullet+}$). α -TOH $^{\bullet+}$ rapidly loses a proton to form the neutral radical (α -TO $^{\bullet}$), which is then immediately oxidized by one-electron at the electrode surface to form the diamagnetic cation (α -TO $^+$).

Scheme 5.1: Electrochemically induced transformations of α -tocopherol in CH₃CN or CH₂Cl₂.¹⁹ One resonance structure is displayed for each compound. The counterions for the charged species are the supporting electrolyte anion [PF₆⁻], and the “H⁺” ions likely exist coordinated to the organic solvent (or with trace water). Formal potentials (E_{f}^0) are vs. ferrocene/ferrocene⁺ at 295 ± 2 K.

Although the mechanism occurs in two one-electron steps, only one oxidative peak (E_p^{ox}) is observed during cyclic voltammetry experiments because the second electron transfer step occurs at a lower (more negative) potential than the first electron-transfer step.²³ α -TOH can be quantitatively regenerated from α -TO⁺ on the milliseconds and hours time scales by applying a reducing potential to the electrode surface. The reaction in Scheme 5.1 is given as an ECE mechanism, where E represents an electron transfer at an electrode surface and C represents a homogeneous chemical step. However, it is uncertain whether the second electron-transfer step occurs via a heterogeneous electron transfer as given in Scheme 5.1 or by a homogeneous disproportionation mechanism.^{25,35} It has been found that chemical oxidation of α -TOH with 2 mol equivalents of NO⁺ leads to α -TO⁺ in 100% yield (eq 5.3).^{20,28}

The free proton that is shown in Scheme 5.1 and eq 5.3 is released from the hydroxyl group during the forward oxidation step.^{16,17,19} It has been assumed that the same proton is involved in protonating the phenoxyl when the diamagnetic cation (α -TO⁺) is reduced back to the starting material via the phenoxyl radical (α -TO[•]).¹⁹⁻²⁹ If a dry organic soluble acid such as CF₃SO₃H or CF₃COOH is added to the organic solvent, the lifetime of the radical cation (α -TOH^{•+}) increases substantially, resulting in the electrochemical oxidation occurring via one-electron (rather than two electrons when the oxidation is conducted in the absence of added acid).^{17,19,21} Spectroscopic experiments (EPR, UV-vis, and FTIR) have shown that α -TOH^{•+} can survive for at least several minutes at room temperature when produced in CH₃CN or CH₂Cl₂ containing strong organic acids.^{17,19,21} However, such a strong acid environment is unlike any conditions that the compound is likely to experience in its natural lipophilic environment.

The electrochemical oxidation of β -Car occurs in two one electron steps to first form the cation radical (β -Car^{•+}) and then the dication (β -Car²⁺) (Scheme 5.2).³⁰⁻³⁴

Detailed voltammetric analysis and quantum mechanical calculations have indicated that the second electron-transfer step most likely occurs at a less positive potential than the first electron-transfer step, although the potentials are very close.³⁴ Thus, cyclic voltammograms of β -Car in dichloromethane only display one forward oxidative peak (E_p^{ox}) associated with a two-electron transfer, and one reverse reductive peak (E_p^{red}) also corresponding to a two-electron transfer. Unlike $\alpha\text{-TO}^+$ which is indefinitely long-lived in dry acetonitrile or dichloromethane, $\beta\text{-Car}^{2+}$ only survives for a few seconds at room temperature in dichloromethane and undergoes a number of homogeneous chemical reactions to form largely unidentified products.³³

Scheme 5.2: Electrochemical oxidation mechanism for β -Car in CH_2Cl_2 .³⁴ Formal potentials (E_f^0) are vs. ferrocene/ferrocene⁺ at $295 \pm 2 \text{ K}$.

5.2 Results and Discussion

5.2.1 Cyclic Voltammetry of $\alpha\text{-TOH}$ and $\beta\text{-Car}$ in Mixed $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ Solutions

A method that enables likely reactions between $\alpha\text{-TO}^+$ and $\beta\text{-Car}$ to be monitored involves preparing bulk solutions of $\alpha\text{-TO}^+$ (that is known to be long lived in low moisture content solvents^{20,22}) and adding them to solutions containing $\beta\text{-Car}$, and monitoring the reaction products voltammetrically. Although $\alpha\text{-TO}^+$ can be prepared by bulk controlled potential electrolysis of $\alpha\text{-TOH}$,¹⁹ a simpler method is via chemical reaction of $\alpha\text{-TOH}$ with 2 mol equivalents of $\text{NO}^+\text{SbF}_6^-$ (eq 5.3).²⁰ Chemical oxidation was deemed preferable to electrochemical oxidation in an electrolysis cell because the chemical oxidation reaction occurs rapidly in 100% conversion and because it is easier to

maintain the reaction solution in a low water content environment.²² However, NOSbF_6 is poorly soluble in CH_2Cl_2 but very soluble in CH_3CN , while $\beta\text{-Car}$ is insoluble in CH_3CN but reasonably soluble in CH_2Cl_2 . Therefore, initial voltammetric experiments were performed by varying the amount of CH_2Cl_2 and CH_3CN to find a ratio where all the reagents were fully soluble.

Figure 5.1 show CVs of $\beta\text{-Car}$ in CH_2Cl_2 at 295 ± 2 K with increasing amounts of CH_3CN added. In pure CH_2Cl_2 , the CV consists of a forward oxidation peak (E_p^{ox}) and a reverse reduction peak (E_p^{red}) separated by approximately 70 mV with a mid potential $([E_p^{\text{ox}} + E_p^{\text{red}}]/2) = +0.1$ V vs. Fc/Fc^+ . The process corresponds to the chemically reversible two-electron oxidation of $\beta\text{-Car}$ into $\beta\text{-Car}^{2+}$ in two indistinguishable one-electron steps.³⁰⁻³⁴ When the CH_2Cl_2 contained 20% CH_3CN , there was a decrease in the chemical reversibility of the oxidation process of $\beta\text{-Car}$ (at a scan rate of 0.1 V s^{-1}), shown by how the anodic (i_p^{ox}) to cathodic (i_p^{red}) peak current ratio ($i_p^{\text{ox}}/i_p^{\text{red}}$) increased to $\gg$ unity. Additional oxidation processes also became evident at potentials more positive than +0.40 V vs. Fc/Fc^+ when CH_3CN was added to the CH_2Cl_2 , associated with reaction products formed due to increased chemical instability (or reactivity) of $\beta\text{-Car}^{2+}$ in the presence of CH_3CN . Furthermore, when the amount of CH_3CN was increased above 40%, the i_p^{ox} value associated with the two-electron oxidation of $\beta\text{-Car}$ decreased substantially, due to insolubility of the $\beta\text{-Car}$.

Figure 5.1: CVs of 1×10^{-3} M β -Car in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K recorded at a 1 mm diameter Pt electrode at a scan rate of 0.1 V s^{-1} with increasing percentages of CH_3CN . Positive current is in the upward direction.

CVs of α -TOH recorded under the same conditions as β -Car are shown in Figure 5.2. The CVs consist of a forward oxidation peak (E_p^{ox}) at approximately +0.50 V vs. Fc/Fc^+ and a reverse reductive peak (E_p^{red}) at between +0.35 to +0.20 V vs. Fc/Fc^+ . The reverse reductive peak shifted to more negative potentials as increasing amounts of CH_3CN were added to the solution.

Figure 5.2: CVs of 1×10^{-3} M α -TOH in CH_2Cl_2 containing 0.2 M Bu_4NPF_6 at 295 ± 2 K recorded at a 1 mm diameter Pt electrode at a scan rate of 0.1 V s^{-1} with increasing percentages of CH_3CN . Positive current is in the upward direction.

The E_{p}^{ox} and $E_{\text{p}}^{\text{red}}$ processes are associated with the forward and reverse reactions, respectively given in Scheme 5.1, and are due to the chemical reversible transformation of α -TOH into $\alpha\text{-TO}^+$.^{19,23} The reason for the wide separation between the E_{p}^{ox} and $E_{\text{p}}^{\text{red}}$ values is because the two one-electron-transfer steps occur at different potentials with a proton-transfer reaction interspersed between the electron transfers (an ECE mechanism) (Scheme 5.1). The potentials given in Scheme 5.1 were estimated by digital simulation techniques at 295 ± 2 K and are difficult to measure experimentally because the coupled proton-transfer reaction shifts the peaks away from the formal potentials.²⁵ The potentials

are also influenced by the temperature and interactions of the phenol (α -TOH) with trace water in the solvent.

By comparing the results in Figures 5.1 and 5.2, it can be seen that α -TOH is more difficult to oxidize than β -Car by approximately +0.40 V (based on the E_p^{ox} values). Therefore, it would be expected that β -Car should be a sufficiently strong reductant to reduce $\alpha\text{-TO}^+$ back to α -TOH (providing protons are available) and thereby be simultaneously oxidized to $\beta\text{-Car}^{2+}$. However, the prediction is uncertain because the second electron-transfer step of α -TOH ($E_{\text{f}(2)}^0$ in Scheme 5.1) occurs at a much lower potential than the first electron-transfer step ($E_{\text{f}(1)}^0$). Therefore, the most reliable way to establish whether the reaction occurs is to prepare a solution of $\alpha\text{-TO}^+/\text{H}^+$ and react it with β -Car and determine if α -TOH and $\beta\text{-Car}^{2+}$ are indeed detected.

Because of the decreased lifetime of $\beta\text{-Car}^{2+}$ in the presence of CH_3CN (Figure 5.1), it was decided to perform the chemical oxidation experiments in CH_2Cl_2 with 20% CH_3CN , which was high enough CH_3CN to enable the NOSbF_6 to fully dissolve but not to interfere in the solubility of β -Car. Figure 5.3 shows variable scan rate CVs of β -Car that were performed in CH_2Cl_2 containing 20% CH_3CN . It can be observed in Figure 5.3 that, as the scan rate approaches 5 V s^{-1} , the processes at potentials $>+0.40 \text{ V vs. Fc/Fc}^+$ decrease in intensity while simultaneously the reverse reduction peak at $\sim+0.05 \text{ V vs. Fc/Fc}^+$ associated with the reduction of $\beta\text{-Car}^{2+}$ back to β -Car increases in magnitude. Therefore, the variable scan rate CV experiments indicate that the lifetime of $\beta\text{-Car}^{2+}$ is relatively short ($\leq 1 \text{ s}$) at $295 \pm 2 \text{ K}$ in CH_2Cl_2 containing 20% CH_3CN , since a scan rate of at least 5 V s^{-1} is needed to obtain an $i_p^{\text{ox}}/i_p^{\text{red}}$ ratio equal to unity.

Figure 5.3: CVs of 1×10^{-3} M β -Car in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) containing 0.2 M Bu_4NPF_6 at 295 ± 2 K recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. The current has been normalized by multiplying by $\nu^{-0.5}$.

5.2.2 Measuring the Half-Life of $\beta\text{-Car}^{2+}$ at Varying Temperatures in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1)

The experiments shown in Figure 5.3 indicated that the lifetime of $\beta\text{-Car}^{2+}$ is relatively short at room temperature. In order to increase the lifetime of $\beta\text{-Car}^{2+}$ and thereby make it easier to detect after reacting $\beta\text{-Car}$ with $\alpha\text{-TO}^+/\text{H}^+$, experiments were performed at low temperatures by reacting $\beta\text{-Car}$ with 2 mol equivalents of NOSbF_6 in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) (eq 5.4) and measuring the decay of $\beta\text{-Car}^{2+}$ using square-wave voltammetry. The experiments were performed by adding 1 mL of a CH_3CN solution

containing NOSbF_6 into 4 ml of a CH_2Cl_2 solution containing 1×10^{-3} M $\beta\text{-Car}$ (and 0.2 M Bu_4NPF_6). Using a Pt working electrode, square-wave voltammetry was performed at a scan rate of 0.1 V s^{-1} with a step potential of 5×10^{-3} V and pulse amplitude of 2×10^{-2} V after the addition of NOSbF_6 , and current values were recorded at a one minute interval with polishing done after each scan. Multiple square-wave voltammograms were performed until the peak associated with the reduction of $\beta\text{-Car}^{2+}$ could not be detected. The same experiment was carried out at varying temperatures to determine a suitable condition to perform the reaction between $\alpha\text{-TO}^+$ and $\beta\text{-Car}$.

There are several studies that have measured the decay of neutral $\beta\text{-Car}$ in a variety of matrixes that have suggested that the reactions do follow first-order kinetics.³⁷⁻⁴⁰ However, the reaction products are complicated and it is possible that several competing pathways are involved.

The first order rate equation can be written as follows:

$\text{Rate} = k [A]$, where $[A]$ is the concentration of the compound.

Differentiating the equation with respect to time will give the new equation:

$$-\frac{d[A]}{dt} = k[A]$$

Integrating the concentration from the initial to final over the time from 0 to t gives the following form:

$$\int_{[A]_0}^{[A]} \frac{1}{[A]} d[A] = -k \int_0^t dt$$

$$\ln[A] - \ln[A]_0 = -kt$$

$$\ln \frac{[A]}{[A]_0} = -kt$$

Therefore, the slope of a plot of $\ln \frac{[A]}{[A]_0}$ vs. t will give the k value. The half-life can be

calculated from the equation: $t_{1/2} = \frac{\ln 2}{k}$

First-order kinetic plots obtained by observing the voltammetric decay of $\beta\text{-Car}^{2+}$ are given in Figures 5.4 and 5.5.

Figure 5.4: First-order kinetic plots of the decomposition of 1×10^{-3} M $\beta\text{-Car}^{2+}$ produced by reacting $\beta\text{-Car}$ with 2 mol equivalents of NOSbF_6 in dichloromethane:acetonitrile (4:1) containing 0.2 M Bu_4NPF_6 at 208 K, 213 K and 218 K. $A = \beta\text{-Car}^{2+}$, which was measured by square wave voltammetry.

Figure 5.5: First-order kinetic plots of the decomposition of 1×10^{-3} M $\beta\text{-Car}^{2+}$ produced by reacting $\beta\text{-Car}$ with 2 mol equivalents of NOSbF_6 in dichloromethane:acetonitrile (4:1) containing 0.2 M Bu_4NPF_6 at 223 K, 233 K and 243 K. $A = \beta\text{-Car}^{2+}$, which was measured by square wave voltammetry.

The estimated half-lives at a range of temperatures are given in Table 5.1.

Temp / K	Half-life / min
208	28.9
213	19.3
218	6.4
223	2.6
233	1.6
243	1.2

Table 5.1: First-order half-life of $\beta\text{-Car}^{2+}$ (Produced according to eq 5.4) measured by square-wave voltammetry in dichloromethane/acetonitrile (4:1) containing 0.2 M Bu_4NPF_6 at various temperatures. Kinetic plots are given in Figures 5.4 and 5.5.

The results in Table 5.1 indicate that there is a substantial increase in the lifetime of $\beta\text{-Car}^{2+}$ as the temperature decreases below 218 K. At temperatures ≥ 243 K, it was found that the loss of $\beta\text{-Car}^{2+}$ occurred at a fast rate that could not be reliably measured by

square-wave voltammetry, which accounts for the relatively large scatter in the kinetic plot at 243 K in Figure 5.5. The exact value of the half-life of $\beta\text{-Car}^{2+}$ at different temperatures is not critical to this work, but based on the results in Table 5.1, it was concluded that, at a temperature of 213 K, the lifetime of $\beta\text{-Car}^{2+}$ was sufficiently long to be able to be easily observed voltammetrically as the major reaction product if formed via the reaction of $\beta\text{-Car}$ with $\alpha\text{-TO}^+$, without interference from other reaction products.

5.2.3 Reacting $\alpha\text{-TO}^+$ with $\beta\text{-Car}$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) at 213 K.

Figure 5.6 shows CVs at a stationary electrode and linear sweep voltammograms (LSVs) using a rotating disk electrode (RDE) of $\beta\text{-Car}$ and $\alpha\text{-TOH}$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) containing 0.2 M Bu_4NPF_6 at 213 K. Because of the very low temperature, both the CV and RDE experiments showed effects of distortion due to the high uncompensated solution resistance. In particular, there was a wide separation between the forward and reverse peaks during CV measurements and the waves detected during LSV measurements at the RDE were drawn out over a relatively wide potential range. Nevertheless, the shapes of the voltammetric waves were acceptable in the sense that the oxidation processes were clearly detected.

Figure 5.6: Voltammograms of 1×10^{-3} M β -Car or α -TOH in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) containing 0.2 M Bu_4NPF_6 at 213 K. CV experiments were performed in a stationary solution at a 1 mm diameter planar Pt electrode at a scan rate of 0.1 V s^{-1} , while RDE experiments were performed at a 3 mm diameter planar Pt RDE with a rotation speed of 2000 rpm at a scan rate of 0.05 V s^{-1} .

When CV experiments are performed at stationary electrodes, the oxidation state of the electroactive molecules present in the bulk solution can sometimes be difficult to determine, because the position of where zero current flow is observed can depend on where the potential scan is first commenced. In contrast, RDE experiments or measurements at microelectrodes always allow the position of zero current flow to be determined because new species from the bulk solution are continually being refreshed at the electrode surface. Therefore, the LSV-RDE experiments were performed as a method for determining the oxidation state of the molecules in solution immediately after the chemical oxidation experiments (reacting $\alpha\text{-TO}^+/\text{H}^+$ with $\beta\text{-Car}$) were performed.

Figure 5.7: Linear sweep voltammogram of 1×10^{-3} M $\beta\text{-Car}$ plus 1×10^{-3} M $\alpha\text{-TOH}$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) containing 0.2 M Bu_4NPF_6 at 213 K and recorded at a scan rate of 0.05 V s^{-1} at a 3 mm diameter planar Pt RDE with a rotation speed of 2000 rpm.

Figure 5.7 shows a LSV-RDE experiment when equal amounts of neutral $\alpha\text{-TOH}$ and $\beta\text{-Car}$ are present in solution, with the voltammetric scan in the positive potential direction. At potentials $< 0 \text{ V vs. Fc/Fc}^+$, no Faradaic current flows because the potential is not sufficiently high to oxidize $\beta\text{-Car}$ or $\alpha\text{-TOH}$. As the potential reaches $+0.05 \text{ V vs. Fc/Fc}^+$, $\beta\text{-Car}$ begins to be oxidized and the current increases. When only $\beta\text{-Car}$ is present in solution, a plateau is reached at approximately $+0.50 \text{ V vs. Fc/Fc}^+$ due to the convective-diffusion limited current (Figure 5.6). However, in the presence of $\alpha\text{-TOH}$, the current continues to rise due to the further oxidation of $\alpha\text{-TOH}$ at more positive potentials

(Figure 5.7). It can be observed in Figure 5.7 that the oxidation process for α -TOH appears to shift to more positive potentials compared to when only α -TOH is present in solution (Figure 5.6). The reason for the apparent shift in potential most likely relates to a homogeneous reaction between α -TO $^+$ /H $^+$ and β -Car in the bulk solution, which regenerates α -TOH, and which then undergoes oxidation again (eq 5.5).

Figure 5.8 shows an LSV-RDE experiment that was commenced within 30 s of adding a solution of α -TO $^+$ /H $^+$ to a solution of β -Car at 213 K.

Figure 5.8: Linear sweep voltammogram obtained immediately (within 1 min) after combining 1×10^{-3} M β -Car with 1×10^{-3} M α -TO $^+$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) containing 0.2 M Bu_4NPF_6 at 213 K and recorded at a scan rate 0.05 V s^{-1} at a 3 mm diameter planar Pt RDE with a rotation speed of 2000 rpm.

The experiment was performed by first reacting α -TOH with 2 mol equivalents of NO^+ in CH_3CN at 233 K to produce α -TO $^+$ /H $^+$ (eq 5.3). Then 1 ml of the α -TO $^+$ /H $^+$ solution was added to 4 ml of β -Car in CH_2Cl_2 at 213 K, so that the concentration of both species was equal to 1×10^{-3} M. The voltammogram in Figure 5.6 shows that the position of zero current flow is between +0.20 and +0.40 V vs. Fc/Fc^+ . Therefore, it can be concluded that vitamin E exists in its neutral form (α -TOH) and β -Car has been oxidized to its dication ($\beta\text{-Car}^{2+}$), according to the forward reaction in eq 5.5. The H $^+$ given in eq 5.5 is released during the initial oxidation of α -TOH by 2 $\text{NO}^+\text{SbF}_6^-$ and most likely

exists in solution bound to trace water or to the solvent molecules, and therefore, does not need to come from any other source.²²

The kinetic data in Table 5.1 indicates that $\beta\text{-Car}^{2+}$ survives at 213 K for a much longer time than the time scale of the voltammetric experiment in Figure 5.8 (the LSV-RDE scan was completed in < 1 min of mixing the solution of $\alpha\text{-TO}^+$ and $\beta\text{-Car}$); therefore, the voltammetric data are not affected by the decay of $\beta\text{-Car}^{2+}$. Based on the magnitude of the limiting current values in Figure 5.8 and comparing them to the limiting current values recorded for equivalent concentrations of the individual neutral compounds under identical conditions (Figure 5.6), it is estimated that the transformation in eq 5.5 occurs in a very high yield ($>90\%$).

Figure 5.9: Background subtracted UV-vis spectra obtained in solutions of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) in an approximately 0.5 mm path length quartz cell at 295 ± 2 K: (—) 1×10^{-3} M $\alpha\text{-TOH}$, (---) 1×10^{-3} M $\beta\text{-Car}$, and (...) the products of the reaction between 1×10^{-3} M $\alpha\text{-TO}^+/\text{H}^+$ and 1×10^{-3} M $\beta\text{-Car}$ according to eq 5.5.

The products of the reaction between $\alpha\text{-TO}^+/\text{H}^+$ and $\beta\text{-Car}$ were also measured by UV-vis spectroscopy after the solution was warmed to room temperature. Figure 5.9 (red line) shows the UV-vis spectrum of 1×10^{-3} M $\alpha\text{-TOH}$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) which displays an absorbance at 295 nm with $\epsilon = 3.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$.^{28,41} The UV-vis spectrum of solutions containing $\beta\text{-Car}$ obtained under the same conditions and concentration as $\alpha\text{-TOH}$ displayed a strong absorbance at 276 nm and very strong absorbances between 360 and 560 nm (Figure 5.9, blue dashed line). The spectra in

Figure 5.9 indicate that solutions containing β -Car absorb much more strongly than α -TOH over all wavelengths <600 nm. An additional UV-vis spectrum of β -Car at lower concentration is given in Figure 5.10.

Figure 5.10: UV-vis spectrum of 3×10^{-4} M β -Car in a background subtracted solution of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1) obtained in an approximately 0.5 mm pathlength quartz cell at 295 ± 2 K.

The black dotted line in Figure 5.9 is the UV-vis spectrum obtained after α - TO^+/H^+ was reacted with β -Car at 213 K and then the solution allowed to warm to room temperature. The spectrum of the reaction products in Figure 5.9 (dotted black line) supports the reaction mechanism given in eq 5.5, where the α -TOH is regenerated from α - TO^+/H^+ and all of the β -Car converted into $\beta\text{-Car}^{2+}$. An absorbance band was detected at 295 nm (Figure 5.9, black dotted line) with a similar shape and intensity as expected for α -TOH (Figure 5.9, red line), taking into account that the background absorbance readings at all wavelengths <600 nm are much higher when β -Car and its reaction products are also present. Furthermore, the very intense bands associated with neutral β -Car between 360 and 560 nm are missing from the spectrum of the reaction products, indicating that all of the β -Car had reacted with α - TO^+/H^+ , thereby supporting the quantitative reaction of α - TO^+/H^+ with β -Car in a two-electron process. At the higher temperature, the $\beta\text{-Car}^{2+}$ is unstable/reactive and further reacts/decomposes to form other unknown products.

5.3 Conclusion

The results in this study have proven that β -Car is able to regenerate α -TOH from α -TO⁺/H⁺ in a $-2e^-$ homogeneous reaction to form β -Car²⁺ in high yield. The reaction was performed at low temperatures in order to stabilize the β -Car²⁺ for sufficiently long time to allow its unequivocal identification using linear sweep voltammetry at a rotating disk electrode. Future experiments at higher temperatures to determine the rate of reaction of α -TO⁺/H⁺ and β -Car are possible using stopped flow measurements combined with UV-vis spectrophotometric detection, since both β -Car and α -TO⁺ are long-lived in separate solutions (provided the moisture contents of the solvents are low).

5.4 References

1. Biochemistry of Lipids, Lipoproteins and Membranes, 4th ed.; Vance, D. E., Vance, I. E., Eds.; Elsevier: Amsterdam, 2002.
2. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201.
3. Bowry, V. W.; Ingold, K. U. *Acc. Chem. Res.* **1999**, *32*, 27–34.
4. Traber, M. G.; Atkinson, J. *Free Radical Biol. Med.* **2007**, *43*, 4–15.
5. Azzi, A. *Free Radical Biol. Med.* **2007**, *43*, 16–21.
6. Boscoboinik, D.; Szewczyk, A.; Hensey, C.; Azzi, A. *J. Biol. Chem.* **1991**, *266*, 6188–6194.
7. Tasinato, A.; Boscoboinik, D.; Bartoli, G.-M.; Maroni, P.; Azzi, A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12190–12194.
8. Azzi, A.; Stocker, A. *Prog. Lipid Res.* **2000**, *39*, 231–255.
9. Melton, L. *New Scientist* **2006**, *191*, 40–43.
10. Brigelius-Flohé, R.; Davies, K. J. A. *Free Radical Biol. Med.* **2007**, *43*, 2–3.
11. Koyama, Y. *J. Photochem. Photobiol.* **1991**, *B9*, 265–280.
12. Burton, G. W.; Ingold, K. U. *Science* **1984**, *224*, 569–573.
13. Böhm, F.; Edge, R.; Land, E. J.; McGarvey, D. J.; Truscott, T. G. *J. Am. Chem. Soc.* **1997**, *119*, 621–622.
14. Valgimigli, L.; Lucarini, M.; Pedulli, G. F.; Ingold, K. U. *J. Am. Chem. Soc.* **1997**, *119*, 8095–8096.
15. Edge, R.; Land, E. J.; McGarvey, D.; Mulroy, L.; Truscott, T. G. *J. Am. Chem. Soc.* **1998**, *120*, 4087–4090.
16. Marcus, M. F.; Hawley, M. D. *Biochim. Biophys. Acta* **1970**, *201*, 1–8.
17. Svanholm, U.; Bechgaard, K.; Parker, V. D. *J. Am. Chem. Soc.* **1974**, *96*, 2409–2413.
18. Webster, R. D. *Electrochem. Commun.* **1999**, *1*, 581–584.

19. Williams, L. L.; Webster, R. D. *J. Am. Chem. Soc.* **2004**, *126*, 12441–12450.
20. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D. *J. Org. Chem.* **2005**, *70*, 10466–10473.
21. Wilson, G. J.; Lin, C. Y.; Webster, R. D. *J. Phys. Chem. B* **2006**, *110*, 11540–11548.
22. Lee, S. B.; Willis, A. C.; Webster, R. D. *J. Am. Chem. Soc.* **2006**, *128*, 9332–9333.
23. Webster, R. D. *Acc. Chem. Res.* **2007**, *40*, 251–257.
24. Peng, H. M.; Webster, R. D. *J. Org. Chem.* **2008**, *73*, 2169–2175.
25. Yao, W. W.; Peng, H. M.; Webster, R. D.; Gill, P. M. W. *J. Phys. Chem. B* **2008**, *112*, 6847–6855.
26. Peng, H. M.; Choules, B. F.; Yao, W. W.; Zhang, Z.; Webster, R. D.; Gill, P. M. W. *J. Phys. Chem. B* **2008**, *112*, 10367–10374.
27. Yao, W. W.; Peng, H. M.; Webster, R. D. *J. Phys. Chem. C* **2009**, *113*, 21805–21814.
28. Chen, S.; Peng, H. M.; Webster, R. D. *Electrochim. Acta* **2010**, *55*, 8863–8869.
29. Yao, W. W.; Lau, C.; Hui, Y.; Poh, H. W.; Webster, R. D. *J. Phys. Chem. C* **2011**, *115*, 2100–2113.
30. Jeevarajan, J. A.; Jeevarajan, A. S.; Kispert, L. D. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 1757–1765.
31. Jeevarajan, J. A.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. *J. Phys. Chem.* **1996**, *100*, 5637–5641.
32. Kispert, L. D.; Gao, G.; Deng, Y.; Konovalov, V.; Jeevarajan, A. S.; Jeevarajan, J. A.; Hand, E. *Acta Chem. Scand.* **1997**, *51*, 572–578.
33. Liu, D. Z.; Gao, Y. L.; Kispert, L. D. *J. Electroanal. Chem.* **2000**, *488*, 140–150.
34. Hapiot, P.; Kispert, L. D.; Konovalov, V. V.; Saveant, J.-M. *J. Am. Chem. Soc.* **2001**, *123*, 6669–6677.

35. Costentin, C. *Chem. Rev.* **2008**, *108*, 2145–2179.
36. Hui, Y.; Webster, R. D. *Anal. Chem.* **2011**, *83*, 976–981.
37. Mortensen, A.; Skibsted, L. H. *J. Agric. Food. Chem.* **2000**, *48*, 279–286.
38. Wache, Y.; Bosser-DeRatuld, A.; Lhuguenot, J.-C.; Belin, J.-M. *J. Agric. Food. Chem.* **2003**, *51*, 1984–1987.
39. Dutta, D.; Dutta, A.; Raychaudhuri, U.; Chakraborty, R. *J. Food. Eng.* **2006**, *76*, 538–546.
40. Ferreira, J. E. M.; Rodriguez-Amaya, D. B. *J. Food. Sci.* **2008**, *78*, C589–C594.
41. Naqvi, K. R.; Li, H.; Melø, T. B.; Zhang, Y.; Webster, R. D. *J. Phys. Chem. A* **2010**, *114*, 10795–10802.

Chapter 6

The Role of Low Levels of Water in the Electrochemical Oxidation of α -Tocopherol (Vitamin E) in Acetonitrile

6.1 Introduction

The electrochemical oxidation reaction mechanism of α -tocopherol has previously been thoroughly studied.¹⁻⁶ However, the role of water in the electrochemical oxidation of α -tocopherol had not been explored in detailed. Therefore, in this Chapter, cyclic voltammetry was used to investigate the role that low levels of water have in the oxidative behavior of α -tocopherol (similar to the experiments performed on the reduction of retinal).

Many naturally occurring phenols have specific functions in biological systems. They may behave in a harmful way such as acting as endocrine disruptive chemicals,⁷ or in a beneficial way such as acting as antioxidants and preventing free radical damage of cell membranes.⁸⁻¹⁰ Electrochemical methods are a useful way to examine electron transfer reactions of phenolic compounds where the oxidative properties of the compounds are important.^{11,12} Since many naturally occurring phenols exist within bilayer membranes, the effects of low levels of water present in the hydrophobic environment may affect their oxidative or antioxidant properties.

Vitamin E is a lipid soluble phenolic compound and its exact function has taken on a degree of controversy in recent years.^{10,13} The majority of studies have argued that its sole function is to act as a chain breaking antioxidant (essentially a sacrificial compound)⁸⁻¹⁰ while other reports have proposed its main purpose is as a cellular signalling molecule.¹³⁻¹⁶ It is a general property of all phenolic compounds that they are able to act as antioxidants, where their labile hydroxyl hydrogen atom can be donated to reactive free radicals, and hence terminate radical propagation reactions. α -Tocopherol (α -

TOH), the most biologically active form of vitamin E, displays some other unusual electrochemical properties that are not observed on the other phenols.^{1-6,12,17-24} It has been proposed that these properties may be important for its non-antioxidant biological functions (should they really occur).^{20,21}

α -TOH is initially oxidised by one-electron to form a radical cation, α -TOH^{•+}. The radical cation, α -TOH^{•+}, rapidly loses a proton to form the neutral radical (α -TO[•]), which is immediately further oxidised by one-electron to form the diamagnetic cation (α -TO⁺) (Scheme 6.1).^{2,17,18,21} It is known that the initial one-electron oxidation to form the radical cation occurs by a consecutive mechanism, rather than a concerted proton-coupled electron transfer which has been shown to occur for a number of other phenols.²⁵⁻³³ This is because the radical cation has been positively identified by a range of spectroscopic techniques.^{2,18,20,34} It is uncertain whether the second electron transfer step occurs heterogeneously at the electrode surface as shown in Scheme 6.1 for an ECE process, or whether it occurs by a homogeneous disproportionation reaction (DISP).^{5,33} For some systems it is possible to distinguish the two mechanisms (ECE and DISP) via electrochemical modelling of the voltammetric data,³⁵ although for α -TOH there are additional steps that complicate the detailed modelling of the experiments. For either scenario (ECE or DISP), the oxidation reaction is completely chemically reversible on the cyclic voltammetry (< seconds) and electrolysis (> hours) timescales, provided the solvent only contains low levels of water.^{2-4,21} As the water content of the organic solvent increases, α -TO⁺ is known to undergo a hydrolysis reaction with water. A hemiketal is most likely formed and it further reacts to form a quinone.^{5,6,17,22-24} The difference between α -TOH and most other phenols is that its diamagnetic cation (α -TO⁺, which is often labelled as a phenoxonium ion) is long-lived and reacts relatively slowly with water. There are only a few other examples of phenols that can form long-lived phenoxonium cations,³⁶⁻⁴⁰ with most reacting on the μ s timescales.^{41,42}

Scheme 6.1: Electrochemically induced transformations of α -tocopherol in CH_3CN or CH_2Cl_2 .^{3,17,18} One resonance structure is displayed for each compound. The counter ions for the charged species are the supporting electrolyte anions $[\text{PF}_6^-]$, and the “ H^+ ” ions likely exist coordinated to the organic solvent (or with trace water).

Previous voltammetric experiments were performed on the γ - and δ -tocopherols, whose phenoxonium cations are more reactive with water.^{5,20} They differ from α -TOH in their degree of methylation of the aromatic ring. Digital simulation techniques were used to estimate the rate of hydrolysis of $\gamma\text{-TO}^+$ and $\delta\text{-TO}^+$ in acetonitrile containing background ($\sim 20\text{--}50\text{ mM}$) levels of water.⁵ Because $\alpha\text{-TO}^+$ reacts more slowly with water (compared to $\gamma\text{-TO}^+$ and $\delta\text{-TO}^+$), it is necessary to add more water to the solvent in order to detect the hydrolysis reaction on the cyclic voltammetric time-scale. Therefore, in this Chapter, cyclic voltammetry experiments were conducted on α -TOH over a range of water concentrations which were carefully controlled and quantified using Karl Fischer (KF) titrations. Digital simulations of the voltammetric data were then used to estimate the rate constant of the hydrolysis reaction of $\alpha\text{-TO}^+$.

6.2 Results and Discussion

6.2.1 Method for Preparing Solutions for Electrochemical Measurements

Electrochemical experiments with accurately controlled water concentrations are difficult to perform because there are many ways that water can enter the electrochemical cell. Therefore, careful calibration of experiments are required. To prevent moisture present in the reagents from affecting the experimental results, drying was carried out on the solvent and electrolyte prior to the experiment. Freshly dried molecular sieves were added to the CH_3CN solvent, which was left to stand for 24 h with occasional swirling to

facilitate the trapping of water molecules.⁴³⁻⁴⁵ KF coulometric titrations were carried out on the solvent to determine the water content before and after the drying process. Electrochemical and volumetric glassware were pre-heated at 373 K for a minimum period of 2 h before commencing the experiments and plastic coverings for the electrochemical cells and volumetric flask caps were pre-warmed at 323 K.

The preparation of stock solutions of α -TOH and measurement of reagents were conducted in a 30% relative humidity controlled chamber. Approximately 1 ml of the dried CH_3CN solvent was removed using a syringe, weighed and subjected to KF titration to determine the initial moisture content. The correct mass of Bu_4NPF_6 (electrolyte) was weighed into three separate 10 ml volumetric flasks (Flasks A, B and C) and dried at an elevated temperature of 413 K. Each flask was used to prepare 0.2 M electrolyte concentration when diluted with a solvent after the drying process. In a separate 5 ml volumetric flask (Flask D), the correct quantity of Bu_4NPF_6 was added to produce a 0.5 M electrolyte concentration. The electrolyte in Flask D was then dissolved with dry CH_3CN to give a solution for the salt bridge in the reference electrode. 0.25 mmol (107.68 mg) of α -TOH was weighed into a 25 ml volumetric flask and dissolved with dry CH_3CN to produce a 10 mM stock solution. 2 ml of the stock solution was then added to Flasks A, B and C, followed by dilution with dried CH_3CN to obtain test solutions containing 2×10^{-3} M of α -TOH. A KF titration was performed on the solution in Flask C to establish changes in the water content due to the preparatory work performed in the humidity chamber.

Two glass electrochemical cells (Cells A and B) were fitted with an inert gas bubbler and left to cool under an argon (Ar) atmosphere. The rationale behind the use of Cell B was to assist in determining the change in moisture levels from the course of the preparations, transferring, and purging over the course of the entire experiment, whereas actual voltammetric experiments were conducted in Cell A. As aliquots of water were

added to Cell A, it was important to estimate how much atmospheric water also entered the cell over the time of all the voltammetric experiments (approximately 2 h), hence the need for Cell B. Furthermore, since the KF titration is a destructive technique, it is not possible to directly measure the water content in Cell A without removing some of the solution. This is not desirable because it alters the solution volume and complicates calculations of the water concentration when additional volumes are added. Next, the test solutions in Flasks A and B were transferred, respectively, into Cells A and B and deoxygenated with dry Ar gas for 5 min. KF titration was then carried out on a 1 mL aliquot of the solution in Cell B to determine the change in water concentration due to transferring and purging.

CV scans were conducted at variable scan rates at room temperature (295 ± 2 K) on the test solution in Cell A with increasing water concentrations. The electrode was repolished after each addition of water. The first set of scans contained no added water, with water then added in 20 μ L increments up to 100 μ L (total volume). Each 20 μ L increment of water corresponded to the addition of 0.11 M H₂O. At the end, after a total addition of 100 μ L of ultrapure water, ferrocene was added as an internal reference standard. Finally, a KF titration was carried out on a 1 mL aliquot of the solution in Cell B to determine if there was any significant change in its water concentration compared to that at the start of the experiment. The variable scan rate experiments were repeated three times to ensure that the results obtained were accurate and reproducible.

Solution conditions	Water content / mol L ⁻¹
CH ₃ CN (before drying)	$5 \times 10^{-3} - 50 \times 10^{-3}$
CH ₃ CN (after drying)	$0.24 \times 10^{-3} - 0.37 \times 10^{-3}$
Test solution in Flask C	$10.50 \times 10^{-3} - 11.02 \times 10^{-3}$
Test solution in Cell B (start of the experiment)	$12.29 \times 10^{-3} - 13.62 \times 10^{-3}$
Test solution in Cell B (end of the experiment)	$39.50 \times 10^{-3} - 58.40 \times 10^{-3}$

Table 6.1: Water concentrations measured by Karl Fischer (KF) coulometric titrations for solutions used for electrochemical measurements.

The data in Table 6.1 are the water concentrations of the solutions measured by KF titrations at various intervals. Before drying, the CH₃CN contained 0.005–0.05 M H₂O, which depended on the solvent source and the length of time after the solvent bottle was first opened. After storing the CH₃CN over the molecular sieves, the water content decreased to $<0.5 \times 10^{-3}$ M.⁴³ After adding the solvent to the volumetric flask containing the supporting electrolyte, the water content increased to $\sim 10 \times 10^{-3}$ M (test solution in Flask C), and after adding the solvent/electrolyte into the electrochemical cell, the water content increased to approximately 13×10^{-3} M (test solution in Cell B (start of the experiment)). Storing the solvent/electrolyte in the electrochemical cell under an argon atmosphere over approximately 2 h resulted in the water content increasing to between $40\text{--}60 \times 10^{-3}$ M (test solution in Cell B (end of the experiment)). Because approximately 10 times more water was being deliberately added over the course of the experiments, it was concluded that the error associated with the natural increase of water into the electrochemical cell was approximately 10% (sufficiently low so as not to affect the digital modelling).

Using a pseudo reference electrode (and an internal standard such as ferrocene) it is possible to work with water concentrations of $<1 \times 10^{-3}$ M in CH₃CN during voltammetric measurements.⁴³⁻⁴⁵ However, for these experiments interest was in examining how the presence of water affected the peak potentials; thus a stable reference electrode containing a porous frit was required, which meant that the lowest water concentration obtained was approximately 0.010 M.

6.2.2 CV Measurements on α -TOH with Variable Water Concentrations (0.013 M–0.56 M)

Figure 6.1 shows CVs of 2×10^{-3} M α -TOH in CH₃CN (containing 0.2 M Bu₄NPF₆) at a scan rate of 0.1 V s⁻¹ with variable concentrations of H₂O. With a water content of $13 (\pm 1) \times 10^{-3}$ M, the CV shows an oxidative peak (E_p^{ox}) at approximately

+0.50 V vs. Fc/Fc^+ and a reverse (reductive) peak (E_p^{red}) at approximately +0.20 V vs. Fc/Fc^+ that is split into two peaks at scan rates $< 0.5 \text{ V s}^{-1}$, and converts into one process at faster scan rates. The forward and reverse processes are associated with the chemically reversible transformation of $\alpha\text{-TOH}$ into $\alpha\text{-TO}^+$ according to the mechanism in Scheme 6.1.

Figure 6.1: CVs of $2 \times 10^{-3} \text{ M}$ $\alpha\text{-TOH}$ in CH_3CN containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at a scan rate (ν) of 0.1 V s^{-1} in the presence of varying amounts of water. Current data were scaled by multiplying by $\nu^{-0.5}$. Positive current is in the upward direction. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan.

The reason that there is a wide separation (ΔE_{pp}) between the forward (E_p^{ox}) and reverse (E_p^{red}) processes is because the two one-electron transfer steps occur at different

potentials.^{2-6,17,18,20-24} The E_p^{ox} and E_p^{red} potentials are not easily related to the formal one-electron potentials because the proton transfer reaction interspaced between the electron transfer steps shifts the peak values away from the formal potentials. As the scan rate increases (Figure 6.2), the separation between the forward and reverse processes further increases, which is mainly caused by a relatively slow rate of heterogeneous electron transfer ($k_s = 0.1\text{--}0.3 \text{ cm s}^{-1}$) of one or more of the electron transfer steps, rather than due to the effects of uncompensated solution resistance ($\sim 10^2 \Omega$).⁵

The reason for the appearance of two closely-spaced reductive peaks between +0.10 and +0.30 V vs. Fc/Fc⁺ at slow scan rates in Figure 6.1 (at a water concentration of 0.013 M) is presently not clear, but the two peaks are only observed if the water content is <0.02 M. At higher water concentrations or faster scan rates or lower temperature, only one process is observed. The two closely-spaced reductive peaks are always observed when slow scan rate (<0.5 V s⁻¹) CV experiments on α -TOH are performed in dichloromethane,^{3,5} because dichloromethane naturally has much lower water content than CH₃CN.⁴³⁻⁴⁵

Figure 6.2: CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.013 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Current data were scaled by multiplying by $\nu^{-0.5}$. Positive current is in the upward direction and the starting and finishing potential is -1 V vs. Fc/Fc^+ . The forward scan in the positive direction shows the oxidation peak of α -TOH to the phenoxonium cation ($\alpha\text{-TO}^+$) via $2e^-/\text{H}^+$ process while the reverse scan in the negative direction shows the reduction peak of the phenoxonium cation ($\alpha\text{-TO}^+$) back to the starting material of α -TOH.

When the water content of the CH_3CN is $< \sim 50 \times 10^{-3}$ M, there is no voltammetric evidence of the hemiketal ($\alpha\text{-TOQ(OH)}$) that can be formed via the reaction of $\alpha\text{-TO}^+$ with water (Scheme 6.2), since the reaction occurs too slowly to be detected on the CV

time-scale. As the water content of the solvent increases, the lifetime of the α -TO⁺ decreases; thus the presence of the hemiketal becomes evident as a reductive peak at approximately $-0.3 (\pm 0.2)$ V vs. Fc/Fc⁺ when the scan was first performed in the positive potential direction so as to oxidise α -TOH to α -TO⁺. It can be observed in Figure 6.1 that as more water is added, the peak for the reduction of the α -TO⁺ back to α -TOH at $\sim +0.20$ V vs. Fc/Fc⁺ diminishes in size, while concomitantly the peak associated with the reduction of the hemiketal at $\sim -0.3 (\pm 0.2)$ V vs. Fc/Fc⁺ becomes larger.

Scheme 6.2: Reaction between the diamagnetic cation (α -TO⁺) and water to form a hemiketal (α -TOQ(OH)).

It is difficult to obtain spectroscopic data to confirm the existence of the hemiketal shown in Scheme 6.2 because such compounds are difficult to isolate from solution as they rapidly convert into the quinones (α -TOQ) in the presence of small amounts of acid.⁴⁶⁻⁵¹ The assignment of the process at $-0.3 (\pm 0.2)$ V vs. Fc/Fc⁺ as due to the hemiketal was based on previous electrolysis experiments.^{6,23} Controlled potential electrolysis of α -TOH and several other phenols with similar structures in CH₃CN containing ~ 0.05 – 0.1 M H₂O resulted in the formation of the species at $-0.3 (\pm 0.2)$ V vs. Fc/Fc⁺ that survived in the bulk solution for several minutes.⁶ It was found that applying a reductive potential ($> -0.3 (\pm 0.2)$ V vs. Fc/Fc⁺) resulted in the oxidised species partly converting back to the starting material. Thus, it was reasoned that the most likely compound for the process at $-0.3 (\pm 0.2)$ V vs. Fc/Fc⁺ was the hemiketal, since any ring-opened structure (such as the quinone (α -TOQ)) was unlikely to be able to be converted back to the ring-closed form simply by applying a reducing potential.

The CVs in Figure 6.1 demonstrate that the potential of the oxidation peak (E_p^{ox}) shifts to less positive potentials as increasing concentrations of water are added to the solvent, which clearly demonstrates that α -TOH is undergoing an interaction with relatively low levels of water in the solvent. In order to narrow down the possible reasons for the shift in potential, additional electrochemical experiments were conducted on α -TOH.

6.2.3 Cyclic Voltammetry of α -TOH in the Presence of Water

Interactions between α -TOH and small quantities of weak bases in methanol have previously been reported.^{52,53} The oxidation peak of α -TOH was observed to shift to less positive potentials as small amounts of bases were added to the methanol solutions, by a similar magnitude observed in this study (0.2-0.4 V).^{52,53} It is likely that the interaction between the coordinating solvent/base occurs at the hydroxyl proton on α -TOH. However, the important question is whether the addition of water can cause deprotonation of the α -TOH. In CH_3CN , H_2O is able to act as a base,⁵⁴ but whether it can cause complete deprotonation of α -TOH (which is weakly acidic in aqueous micellar solutions)⁵⁵ is questionable. When a very strong base, such as Et_4NOH , is added to the CH_3CN , α -TOH undergoes complete deprotonation to form the phenolate anion, $\alpha\text{-TO}^-$, which is much more easily oxidised at approximately -0.90 V vs. Fc/Fc^+ .^{1,19} The shift in oxidative peak potential of α -TOH to less positive potentials when water is added to the CH_3CN is in the order of ≤ 0.4 V. This negative potential shift is substantially less than that occurs during complete deprotonation to form $\alpha\text{-TO}^-$, where the shift occurs by approximately 1.5 V.

The shift in oxidation peak potential when water is added to the CH_3CN solutions is consistent with a hydrogen-bonding mechanism, where the H_2O interacts weakly with the hydroxyl proton on the α -TOH. In this case it is sensible that the oxidation peak would shift to less positive potentials with increasing hydrogen bonding, because it would result in a weakening of the phenolic oxygen–hydrogen bond, thereby giving the phenol

some of the characteristics of a phenolate (which is much easier to oxidise than phenols). It has been reported that hydrogen-bonding of bases is greatly enhanced in acetonitrile compared to that in water.⁵⁶ Intramolecular hydrogen-bonding of phenols has been reported to shift their oxidation potential to less positive potentials.^{25-27,57}

A similar hydrogen-bonding effect has been observed during the reduction of quinones in organic solvents which display a large shift in peak potential (especially for the second one electron transfer), due to hydrogen-bonding between the anion radical and dianion with trace water.^{44,45,58,59} However, in the case of quinones, the shift in the reduction peak potentials occurs to less negative potentials as more water is added to the solution (rather than less positive potentials observed for α -TOH). Nevertheless, the shift in peak potential with increasing water content does not necessarily have to occur in the same potential direction for processes involving reduction and oxidation. Therefore, the direction of the peak potential shift observed in the voltammetric behavior of compounds susceptible to hydrogen-bonding interactions with water needs to be determined on a case-by-case basis for different classes of compounds.

The CVs in Figure 6.1 demonstrate that the presence of relatively small amounts of water in the solvent is sufficient to shift the peak potential of the oxidation process. Therefore, when reporting the oxidative behavior of phenols in organic solvents, the accurate water content of the solvent should also be reported.

6.2.4 Digital Simulation of CV Measurements on α -TOH with Variable Water Concentrations (0.013 M–0.56 M)

CV data of α -TOH in CH₃CN obtained at variable scan rates in the presence of 0.23 M H₂O is shown in Figure 6.3. The current traces have been normalized by multiplying by $\nu^{-0.5}$ (ν =scan rate).

Figure 6.3: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.233 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

It can be observed in Figure 6.3 that at a scan rate of 0.1 V s^{-1} , the water content is sufficiently high to be able to detect the immediate formation of the hemiketal at approximately $-0.3 (\pm 0.2)$ V vs. Fc/Fc^+ during the reverse scan, after the initial oxidation

of α -TOH on the forward scan. When the scan rate is progressively increased, the peak associated with the hemiketal at $-0.3 (\pm 0.2)$ V vs. Fc/Fc^+ diminishes in size while concomitantly the peak associated with the reduction of $\alpha\text{-TO}^+$ at $0 - +0.2$ V vs. Fc/Fc^+ increases in size, due to the hydrolysis reaction being outrun at faster scan rates.

By performing a number of CVs of α -TOH at various scan rates in the presence of varying amounts of carefully controlled water ($0.013 - 0.56$ M), it was possible to model the electrochemical data by digital simulation techniques in order to obtain the approximate electrochemical potentials and the rate constant associated with the hydrolysis reaction between $\alpha\text{-TO}^+$ and H_2O , which are provided in Tables 6.2 and 6.3. The complete electrochemical mechanism, including the proposed hydrogen-bonding interactions between the starting material and water, is given in Scheme 6.3. The dashed lines in Figure 6.3 are the simulated voltammograms for 0.23 M H_2O . The other simulated voltammograms for different water contents are shown in Figure 6.4, 6.5, 6.6, 6.7 and 6.8. Because of the large number of steps in the mechanism shown in Scheme 6.3, it was not possible to obtain voltammograms that exactly matched the experimental voltammograms over all scan rates and water contents, when one unique set of electrochemical and kinetic parameters were used. The simulated voltammograms that are presented in Figures 6.3–6.8 all use identical parameters to those given in Tables 6.2 and 6.3. The values represent the best average fits for all of the data.

Figure 6.4: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.013 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

Figure 6.5: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.123 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

Figure 6.6: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.343 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

Figure 6.7: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.453 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

Figure 6.8: Experimental CVs of 2×10^{-3} M α -TOH in CH_3CN (with 0.563 M H_2O) containing 0.2 M Bu_4NPF_6 recorded at a 1 mm diameter Pt electrode at varying scan rates (ν). Positive current is in the upward direction. Current data were scaled by multiplying by $\nu^{-0.5}$. The starting potential is -1 V vs. Fc/Fc^+ and the finishing potential is 0 V vs. Fc/Fc^+ on the second scan. The black dotted line (---) shows digital simulations of experimental data according to the mechanism in Scheme 6.3 and parameters in Tables 6.2 and 6.3.

$E^{\circ}_{f(1)}/V$	$E^{\circ}_{f(2)}/V$	$E^{\circ}_{f(3)}/V$	$E^{\circ}_{f(4)}/V$
+0.6	+0.5	+0.075	-0.3 (± 0.2)

Table 6.2: Electrochemical potentials obtained by digital simulation of CV data for the reaction mechanism given in Scheme 6.3. CV data recorded in CH₃CN with 0.2 M n-Bu₄NPF₆ as the supporting electrolyte at a 1 mm diameter Pt electrode at 295 (± 2) K, at scan rates between 0.1 – 20 V s⁻¹ and at water concentrations between 0.013 – 0.56 M. ^a Formal potential vs. Fc/Fc⁺.

Kinetic parameters	Eq 1	Eq2	Eq 3	Eq 4	Eq 5	Eq 6	Eq 7	Eq 8
K_{eq}	10	2.0×10^{-1}	1.4×10^{-4}	1.2×10^{-4}	1	1.0×10^3	1.0×10^3	1.0×10^3
k_f^a	1.0×10^2	1.0×10^2	3.0×10^4	3.0×10^5	11 ± 4	3.0×10^4	1	0.1
k_b^a	10	4.9×10^2	2.5×10^8	2.5×10^9	11 ± 4	30	1.0×10^{-3}	1.0×10^{-4}

Table 6.3: Equilibrium and rate constants obtained by digital simulation of CV data for the reaction mechanism given in Scheme 6.3. CV data recorded in CH₃CN with 0.2 M n-Bu₄NPF₆ as the supporting electrolyte at a 1 mm diameter Pt electrode at 295 (± 2) K, at scan rates between 0.1 – 20 V s⁻¹ and at water concentrations between 0.013 – 0.55 M. Heterogeneous rate constants were estimated to be between 0.1 – 0.3 cm s⁻¹. Diffusion coefficient values were 2.0×10^{-5} cm² s⁻¹ for α -TOH (and its oxidised forms) and 4.0×10^{-5} for H₂O, H⁺ and H₃O⁺. ^a Homogeneous rate constants for the forward (k_f) and back (k_b) reactions have units of s⁻¹ and L mol⁻¹ s⁻¹ for first- and second-order reactions, respectively.

Scheme 6.3: Electrochemical oxidation mechanism of α -tocopherol in CH_3CN studied by cyclic voltammetry over a range of scan rates and water concentrations. Electrochemical, equilibrium and kinetic values associated with the heterogeneous electron transfer and homogeneous chemical steps (Eqs 1–8) are given in Tables 6.2 and 6.3. $\text{R} = (\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3))_3\text{CH}_3$.

The hydrolysis reaction between $\alpha\text{-TO}^+$ and H_2O to form the hemiketal ($\alpha\text{-TOQ(OH)}$) is given in two steps, eq 5 and eq 6 in Scheme 6.3. The first step is the reaction of water at the aromatic ether carbon (eq 5 in Scheme 6.3) followed by the loss of a proton to form the hemiketal (eq 6 in Scheme 6.3). The voltammetric data cannot presently be used to discriminate between the two steps, thus the slower process was arbitrarily assigned to the addition of water (eq 5 in Scheme 6.3). The rate determining step for the reaction of $\alpha\text{-TO}^+$ with water was estimated to be $11 \text{ L mol}^{-1} \text{ s}^{-1}$, which is approximately 5 times slower than that estimated for the reaction between $\gamma\text{-TO}^+$ and $\delta\text{-TO}^+$ with water in acetonitrile.⁵ Eq 7 and eq 8 in Scheme 6.3 (and Table 6.3) are homogeneous reactions of the hemiketal anion radical and hemiketal, respectively.

6.3 Conclusion

CV experiments on $\alpha\text{-TOH}$ in CH_3CN in the presence of varying concentrations of water indicated that $\alpha\text{-TOH}$ most likely undergoes weak hydrogen-bonding interactions with H_2O . This results in the E_p^{ox} -values shifting to less positive potentials as water is added to CH_3CN . Therefore, it may be beneficial to know the precise trace water content of the solvent when interpreting the electrochemical behavior of phenols in organic solvents. Furthermore, the antioxidant activity of phenols has been found to be influenced by kinetic solvent effects (KSE) where hydrogen-bonding of the phenol with the solvent alters the rate of reaction of the phenol with reactive free radicals.^{10,60,61} Therefore, as well as the solvent, the trace water content of the solvent may also be important in determining the antioxidant properties of phenols.

$\alpha\text{-TOH}$ is electrochemically oxidised in CH_3CN to form the diamagnetic cation, $\alpha\text{-TO}^+$, in a chemically reversible $-2e^-/-\text{H}^+$ process. In the presence of water in the CH_3CN , $\alpha\text{-TO}^+$ undergoes a hydrolysis reaction with a rate constant of approximately $11 \text{ L mol}^{-1} \text{ s}^{-1}$. However, in the complete absence of water, or if the water content is considerably less than the concentration of $\alpha\text{-TO}^+$, the diamagnetic cation survives for many hours at

room temperature. It has been shown that the diamagnetic cations of the γ - and δ -tocopherols are less long-lived in solution, reacting with trace water approximately 5 times faster than α -TO⁺.⁵ The reason that γ -TO⁺ and δ -TO⁺ appear much shorter-lived than α -TO⁺ during CV experiments comes from the fact that the trace water content of the solvent is often much higher than the analyte concentration. In contrast, in order to observe the reaction between α -TO⁺ and H₂O on the voltammetric timescale, water must be added to the solvent. Since water is always an impurity in organic solvents, the lifetime of the diamagnetic cations of all of the tocopherols will vary depending on the trace water content of the solvent.

6.4 References

1. Webster, R. D. *Electrochem. Commun.* **1999**, *1*, 581-584.
2. Williams, L. L.; Webster, R. D. *J. Am. Chem. Soc.* **2004**, *126*, 12441-12450.
3. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D. *J. Org. Chem.* **2005**, *70*, 10466–10473.
4. Lee, S. B.; Willis, A. C.; Webster, R. D. *J. Am. Chem. Soc.* **2006**, *128*, 9332-9333.
5. Yao, W. W.; Peng, H. M.; Webster, R. D.; Gill, P. M. W. *J. Phys. Chem. B* **2008**, *112*, 6847-6855.
6. Peng, H. M.; Webster, R. D. *J. Org. Chem.* **2008**, *73*, 2169-2175.
7. Rappoport, Z. *The Chemistry of Phenols*, Wiley: New York, **2004**.
8. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194-201.
9. Bowry, V. W.; Ingold, K. U. *Acc. Chem. Res.* **1999**, *32*, 27-34.
10. Traber, M. G.; Atkinson, J. *Free Radical Biol. Med.* **2007**, *43*, 4-15.
11. Yoshida, K. *Electrooxidation in Organic Chemistry: The Role of Cation Radicals as Synthetic Intermediates*, Wiley: New York, 1983, 174–186.
12. Hammerich, O.; Svensmark, B. In *Organic Electrochemistry*, 3rd Ed.; Lund, H.; Baizer, M. M., Eds.; Marcel Dekker: New York, 1991, chapter 16.
13. Azzi, A. *Free Radical Biol. Med.* **2007**, *43*, 16-21.
14. Boscoboinik, D.; Szewczyk, A.; Hensey, C.; Azzi, A. *J. Biol. Chem.* **1991**, *266*, 6188-6194.
15. Tasinato, A.; Boscoboinik, D.; Bartoli, G. M.; Maroni, P.; Azzi, A. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12190-12194.
16. Azzi, A.; Stocker, A. *Prog. Lipid Res.* **2000**, *39*, 231-255.
17. Marcus, M. F.; Hawley, M. D. *Biochim. Biophys. Acta Gen. Subj.* **1970**, *201*, 1-8.
18. Svanholm, U.; Bechgaard, K.; Parker, V. D. *J. Am. Chem. Soc.* **1974**, *96*, 2409-2413.

19. Nanni Jr, E. J.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481-4485.
20. Wilson, G. J.; Lin, C. Y.; Webster, R. D. *J. Phys. Chem. B* **2006**, *110*, 11540-11548.
21. Webster, R. D. *Acc. Chem. Res.* **2007**, *40*, 251-257.
22. Yao, W. W.; Peng, H. M.; Webster, R. D. *J. Phys. Chem. C* **2009**, *113*, 21805-21814.
23. Chen, S.; Peng, H. M.; Webster, R. D. *Electrochim. Acta* **2010**, *55*, 8863-8869.
24. Yao, W. W.; Lau, C.; Hui, Y.; Poh, H. W.; Webster, R. D. *J. Phys. Chem. C* **2011**, *115*, 2100-2113.
25. Rhile, I. J.; Mayer, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 12718-12719.
26. Rhile, I. J.; Markle, T. F.; Nagao, H.; DiPasquale, A. G.; Lam, O. P.; Lockwood, M. A.; Rotter, K.; Mayer, J. M. *J. Am. Chem. Soc.* **2006**, *128*, 6075-6088.
27. Markle, T. F.; Mayer, J. M. *Angew. Chem. Int. Ed.* **2008**, *47*, 738-740.
28. Costentin, C.; Robert, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **2006**, *128*, 4552-4553.
29. Costentin, C.; Robert, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **2006**, *128*, 8726-8727.
30. Costentin, C.; Louault, C.; Robert, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **2008**, *130*, 15817-15819.
31. Costentin, C.; Louault, C.; Robert, M.; Savéant, J.-M. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 18143-18148.
32. Costentin, C.; Robert, M.; Savéant, J.-M. *Acc. Chem. Res.* **2010**, *43*, 1019-1029.
33. Costentin, C. *Chem. Rev.* **2008**, *108*, 2145-2179.
34. Naqvi, K. R.; Li, H.; Melø, T. B.; Zhang, Y.; Webster, R. D. *J. Phys. Chem. A* **2010**, *114*, 10795-10802.
35. Amatore, C.; Savéant, J.-M. *J. Electroanal. Chem.* **1977**, *85*, 27-46.
36. Dimroth, K.; Umbach, W.; Thomas, H. *Chem. Ber.* **1967**, *100*, 132-141.

37. Speiser, B.; Rieker, A. *J. Chem. Res. Synop.* **1977**, 314-315.
38. Speiser, B.; Rieker, A. *J. Electroanal. Chem.* **1979**, 102, 373-395.
39. Speiser, B.; Rieker, A. *J. Electroanal. Chem.* **1980**, 110, 231-246.
40. Vigalok, A.; Rybtchinski, B.; Gozin, Y.; Koblenz, T. S.; Ben-David, Y.; Rozenberg, H.; Milstein, D. *J. Am. Chem. Soc.* **2003**, 125, 15692-15693.
41. Novak, M.; Glover, S. A. *J. Am. Chem. Soc.* **2004**, 126, 7748-7749.
42. Wang, Y.-T.; Jin, K. J.; Leopold, S. H.; Wang, J.; Peng, H. L.; Platz, M. S.; Xue, J.; Phillips, D. L.; Glover, S. A.; Novak, M. *J. Am. Chem. Soc.* **2008**, 130, 16021-16030.
43. Hui, Y.; Webster, R. D. *Anal. Chem.* **2011**, 83, 976-981.
44. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D. *J. Am. Chem. Soc.* **2009**, 131, 1523-1534.
45. Hui, Y.; Chng, E. L. K.; Chua, L. P.-L.; Liu, W. Z.; Webster, R. D. *Anal. Chem.* **2010**, 82, 1928-1934.
46. Dürckheimer, W.; Cohen, L. A. *Biochem. Biophys. Res. Commun.* **1962**, 9, 262-265.
47. Borchardt, R. T.; Cohen, L. A. *J. Am. Chem. Soc.* **1973**, 95, 8308-8313.
48. Nilsson, A.; Palmquist, U.; Pettersson, T.; Ronlán, A. *J. Chem. Soc. Perkin Trans. I.* **1978**, 696-707.
49. Omura, K. *J. Org. Chem.* **1989**, 54, 1987-1990.
50. Pelter, A.; Elgendy, S. M. A. *J. Chem. Soc., Perkin Trans. I.* **1993**, 1891-1896.
51. Patel, A.; Netscher, T.; Gille, L.; Mereiter, K.; Rosenau, T. *Tetrahedron.* **2007**, 63, 5312-5318.
52. Taguchi, J.; Ohtsuki, S.; Kusu, F. *J. Electroanal. Chem.* **2003**, 557, 91-97.
53. Kotani, A.; Odagiri, M.; Takamura, K.; Kusu, F. *J. Electroanal. Chem.* **2008**, 624, 323-326.

54. Izutsu, K. *Electrochemistry in Non-aqueous Solutions*, Wiley-VCH: Weinheim, 2003, 79–82.
55. Mukai, K.; Tokunaga, A.; Itoh, S.; Kanasaki, Y.; Ohara, K.; Nagaoka, S.; Abe, K. *J. Phys. Chem. B* **2007**, *111*, 652-662.
56. Coetzee, J. F.; Padmanabhan, G. R. *J. Am. Chem. Soc.* **1965**, *87*, 5005-5010.
57. Kanamori, D.; Furukawa, A.; Okamura, T.; Yamamoto, H.; Ueyama, N. *Org. Biomol. Chem.* **2005**, *3*, 1453-1459.
58. Gupta, N.; Linschitz, H. *J. Am. Chem. Soc.* **1997**, *119*, 6384-6391.
59. Quan, M.; Sanchez, D.; Wasylkiw, M. F.; Smith, D. K. *J. Am. Chem. Soc.* **2007**, *129*, 12847-12856.
60. Iwatsuki, M.; Tsuchiya, J.; Komuro, E.; Yamamoto, Y.; Niki, E. *Biochim. Biophys. Acta Gen. Subj.* **1994**, *1200*, 19-26.
61. Valgimigli, L.; Banks, J. T.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1995**, *117*, 9966-9971.

Chapter 7

Summary/Conclusion

Most vitamins are involved in oxidation and/or reduction reactions as part of their mode of operation in complex biological systems. It is very difficult to examine the chemical properties of the compounds in their natural environment, but electrochemical experiments on the isolated compounds under ideal chemical conditions can provide useful information about possible intermediate species that are produced. In this thesis, an electrochemical approach was used to study the reaction mechanisms different forms of vitamin A and the α -tocopherol form of vitamin E in the aprotic solvents CH_3CN and CH_2Cl_2 at Pt and GC electrodes. Experiments were also performed to see how the presence of water in the organic solvents affected the electrochemical mechanisms.

Electrochemical studies were conducted on the series of vitamin A vitamers (β -carotene, retinoic acid, retinol, retinyl palmitate, retinyl acetate and retinal) to determine their oxidation and reduction behavior on different electrode surfaces and in different aprotic solvent systems. The different vitamers displayed different voltammetric behavior, and bulk electrolysis experiments also showed that varying numbers of electrons were involved in each of the vitamer's reduction and oxidation processes. All the vitamers display two reductive processes when experiments were conducted in acetonitrile (CH_3CN) at a GC working electrode, except for β -Car which is insoluble in CH_3CN and unable to be studied. The reductive peak potentials observed during CV experiments varied substantially between the different vitamins and were between -1.10 to -1.90 V vs. Fc/Fc^+ for the first process. The number of electrons transferred during the reduction was found to be either 1 or 2, based on coulometry measured during bulk controlled potential electrolysis. Retinol displayed an unusually small peak current which made detailed study difficult, since it was likely to be decomposing in solution. All species displayed chemically irreversible reductive peaks during cyclic voltammetry measurements except

for retinal, which could be reversibly reduced to its anion radical in a one-electron process. With a change of working electrode from GC to Pt, the reductive response changed. Retinoic acid and retinal are the only two vitamers which displayed reductive peaks while the other compounds do not show any response within the potential window available with the Pt electrode. Similarly, changing the solvent to dichloromethane while using a GC electrode produced a very different reductive response with only β -Car, retinoic acid and retinal showing reductive peaks (due to the narrower potential window available in CH_2Cl_2). The vitamers reductive response in CH_2Cl_2 using a Pt electrode was very similar to that obtained in CH_3CN , with only retinoic acid and retinal displaying a reductive peak.

All of the vitamers could be oxidised in one chemically irreversible process when voltammetric experiments were conducted in CH_3CN using either a GC or Pt electrodes. When the solvent was changed to CH_2Cl_2 , all of the vitamers showed one oxidation process, with only β -Car displaying a chemically reversible voltammetric behavior while the rest were chemically irreversible. For the oxidation process, the number of electrons transferred per molecule measured by coulometry varied from approximately 0.5 for retinyl palmitate and retinyl acetate, and up to 4 electrons for retinal.

A more detailed study on the reduction mechanism of retinal was conducted using a GC working electrode in CH_3CN to understand the possible reaction that retinal undergoes, as it displayed very different initial reductive CV behavior compared to the other vitamers. The results of the study indicated that retinal undergoes a series of heterogeneous electron transfer followed by homogeneous reactions with trace water in the solvent. The temperature, water content of CH_3CN and concentration of retinal plays a role in determining the reductive reaction pathway. Bulk controlled potential electrolysis experiments indicated that the first reduction process of retinal corresponded to the transfer of $1e^-$, which generates a radical anion ($\text{R}^{\bullet-}$). Further reduction at more negative potentials generates a dianion (R^{2-}). Under conditions where the retinal concentration is

high and the water content of the solvent is low, dimerization of the radical anion ($R^{\bullet-}$) is favored and the dimerized product (R_2^{2-}) is formed.

Water was deliberately added to study the effect that different amount of water had on the reaction mechanism of retinal. When the water content increases, hydrolysis reactions, which includes hydrogen-bonding, and decomposition of the dianion of retinal become more dominant over the dimerization steps. It was difficult to study the electrochemical mechanism because the products of the reduction (dimers and hydrolysis products) themselves underwent oxidation at very similar potentials, resulting in overlapping voltammetric peaks. Synthetic scale electrolysis experiments resulted in the generation of a large number of compounds, which were not identified, although NMR and FTIR experiments on the reaction mixture indicated that reduction of the aldehyde group has likely occurred.

β -Car (pro-vitamin A) had previously been extensively studied and was known to undergo an overall $2e^-$ oxidation process. The two $1e^-$ electron processes appear as a single oxidation peak during CV measurements because both processes have very close oxidation potentials. This is very similar to α -TOH (vitamin E) which also undergoes two one-electron oxidation steps yet only a single oxidation peak is observed during CV experiments. However, the oxidation potential of both compounds is different with α -TOH being harder to oxidize compared to β -Car by approximately +0.40 V. The heterogeneous electron transfer oxidation steps of α -TOH are also interspersed with a proton transfer process which is different from the consecutive electron transfer steps of β -Car. A study was conducted to understand the interaction between oxidized α -TOH and β -Car and if oxidized α -TOH could be regenerated from β -Car by a homogeneous electron transfer process.

The results indicated that β -Car was indeed able to regenerate α -TOH from α -TO $^+$ /H $^+$ in a $-2e^-$ homogeneous reaction to simultaneously form β -Car $^{2+}$ in high yield. In

order to observe this reaction, it was necessary to carry out experiments at low temperatures. This helped to stabilize the $\beta\text{-Car}^{2+}$ for a sufficient amount of time to allow its detection using linear sweep voltammetry at a rotating disk electrode due to its shorter lifetime at room temperature.

A detailed study on the influence of water on the reaction mechanism of $\alpha\text{-TOH}$ was performed. Based on previous studies, $\alpha\text{-TOH}$ is electrochemically oxidised in CH_3CN to form the diamagnetic cation, $\alpha\text{-TO}^+$, in a chemically reversible $-2e^-/-\text{H}^+$ process. As water was added to the solvent, the reverse CV scan indicated the presence of an additional reduction peak at a more negative potentials, which was associated with the reduction of an oxidised reaction product. This product is related to the reaction of the diamagnetic cation ($\alpha\text{-TO}^+$) with water to form a hemiketal. In the presence of water in CH_3CN , $\alpha\text{-TO}^+$ undergoes a hydrolysis reaction with a rate constant of approximately $11 \text{ L mol}^{-1} \text{ s}^{-1}$. However, when the water content is considerably less than the concentration of $\alpha\text{-TO}^+$, the diamagnetic cation survives for many hours at room temperature. By varying the concentrations of water in the analyte solution from ultra-dry conditions to much higher levels, it was found that the initial oxidation peak of $\alpha\text{-TOH}$ continually shifted to less positive potentials. The shift in potential was interpreted as due to hydrogen-bonding interactions between the phenolic hydrogen atom and water molecules.

Therefore, when interpreting the electrochemical behavior of $\alpha\text{-TOH}$ in organic solvents, it may be beneficial to know the precise trace water content of the solvent. As the antioxidant activity of phenols has been found to be influenced by kinetic solvent effects (KSE) where hydrogen-bonding of the phenol with the solvent alters the rate of reaction of the phenol with reactive free radicals. Thus, the trace water content of the solvent may be important in determining the antioxidant properties of phenols.

Publications

1. Tan, Y. S.; Webster, R. D. Electron-Transfer Reactions Between the Diamagnetic Cation of α -Tocopherol (Vitamin E) and β -Carotene. *J. Phys. Chem. B* **2011**, *115*, 4244-4250.
2. Tan, Y. S.; Chen, A.; Hong, W. M.; Kan, J. M.; Kwek, E. S. H.; Lim, S. Y.; Lim, Z. H.; Tessensohn, M. E.; Zhang, Y.; Webster, R. D. The Role of Low Level of Water in the Electrochemical Oxidation of α -Tocopherol (Vitamin E) and Other Phenols in Acetonitrile. *Phys. Chem. Chem. Phys.* **2011**, *13*, 12745-12754.
3. Tan, Y. S.; Yue, Y.; Webster, R. D. Competing Hydrogen-Bonding, Decomposition and Reversible Dimerization Mechanisms during the One- and Two-Electron Electrochemical Reduction of Retinal (Vitamin A). *J. Phys. Chem. B* **2013**, *117*, 9371-9379.