Voltammetric studies on vitamin E in organic solvents and within lipid bilayer membranes

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**NANYANG
TECHNOLOGICAL
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**VOLTAMMETRIC STUDIES ON VITAMIN E IN ORGANIC
SOLVENTS AND WITHIN LIPID BILAYER MEMBRANES**

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Chapter 1

General Introduction

1.1 Phenols

1.1.1 General features of the anodic oxidation of phenols

Phenols refer to a series of compounds which have a six-membered aromatic ring and a hydroxyl group (-OH). The electrooxidation of phenols and derivatives have been plentifully investigated. Scheme 1.1 which is termed as a “Scheme of squares” displays the oxidation mechanism of phenols.^{1,2}

Scheme 1.1 Square scheme of oxidation of phenols

In Scheme 1.1, each of the species are linked to one another. The horizontal direction is an electron transfer process and the vertical is a proton transfer process. From left to right, species 1 (neutral phenol) loses one electron to form species 2 (cation radical) which loses one electron to form species 3 (dication) again. In the reverse direction, species 3 (dication) can form species 1 (neutral phenol) by gaining two electrons. At the same time, species 1, 2 and 3 can lose protons to form species 4 (anion), species 5 (neutral phenoxyl radical) and species 6 (phenoxonium cation) respectively. And species 4, 5 and 6 are interrelated by two successive one electron transfers. The type of species formed in this system are dependent on the pH value of the solution, the electrode potential, the electrode material and the solvent.^{1,3} In real systems, the reactions may be more complicated than the processes described in Scheme 1.1 and may involve dimerization and disproportionation.^{1,4-7}

Figure 1.1 shows the cyclic voltammograms for some phenols in the absence or presence of a base.⁸ In aprotic organic solvents without any base, species 1 (phenol) is oxidised to form species 2 (cation radical) at a positive potential by one-electron. Species 2 is easy to deprotonate to species 5 (neutral phenoxyl radical). Then species 5 is further oxidised to species 6 (phenoxonium cation) by one-electron. Because the potential at which species 1 is oxidised to species 5 is less than the potential at which species 5 is oxidised to species 6, there is only one oxidative peak in Figure 1.1 (black lines).⁹

Figure 1.1 Cyclic voltammograms recorded at 293 K at a scan rate of 0.1 V s^{-1} with a 1.6 mm diameter Pt electrode in CH_3CN with 0.25 M Bu_4NPF_6 and 2.0 mM substrate (Black line) The two-electron oxidation of the substituted phenols (PhOH). (Blue line) The one-electron oxidation of the phenolate anions (PhO^-) prepared by the addition of 2 mM Et_4NOH to the same solutions of PhOH . (Red line) The further oxidation of the phenoxyl radicals ($\text{PhO}^\bullet$).⁸ Adapted from *J. Am. Chem. Soc.* **2004**, 126, 12441-12450

In the presence of a base, such as Et₄NOH added to the organic solvents, species 1 (neutral phenol) can easily convert into species 4 (anion) (Equation 1.1). Species 4 is oxidised at a negative potential to form species 5 (neutral phenoxyl radical) (Equation 1.2) [Figure 1.1 (blue lines)].⁸ Species 5 can be further oxidised into species 6 (phenoxonium cation) by one-electron at a more negative potential [Figure 1.1 (red lines)]. Thus, by using different potential ranges, species 5 or 6 can be generated separately.

1.1.2 Disproportionation

Much of the discussion of the electrochemistry of phenols concerns phenoxyl radicals, which are important intermediates in the oxidation of phenols. Phenoxyl radicals with different ring substitution undergo different reactions. Among them, one of the most long-lived phenoxyl radical is 2, 4, 6-tri-tert-butylphenoxyl radical (Scheme 1.2) which can persist in solution and be examined.⁶ If the para substitution is smaller than tert-butyl, such as isopropyl, ethyl or methyl (Scheme 1.2), they easily undergo dimerization and

disproportionation reactions. The disproportionation of 8, 9 and 10 can be observed during electrolysis experiments.

Scheme 1.2 Structures of phenoxyl radicals: (7) 2, 4, 6-tri-tert-butylphenoxyl radical; (8) 2, 6-di-tert-butyl-4-isopropylphenoxyl radical; (9) 2, 6-di-tert-butyl-4-ethylphenoxyl radical; (10) 2, 6-di-tert-butyl-4-methylphenoxyl radical

For example, phenoxyl radical 8 (Scheme 1.2) is susceptible to disproportionation to quinone methide 11 and the starting phenol 12 (Scheme 1.3) during controlled-potential electrolysis.¹⁰ This reaction occurs at intermediate time-scales, so it is too slow to affect the cyclic voltammetry response.

Scheme 1.3 Disproportionation step

1.1.3 Dimerization

Phenoxy radicals may also dimerize to the para-quinone ether. This reaction may occur when the para-substituent is smaller than isopropyl (species 8 in Scheme 1.2) and sterically small groups such as acetyl, pivaloyl and cyano. Kinetic studies show the dimerization of species 9 and 10 (Scheme 1.2) is fast and the dimerization rate constants are about $10^6 \text{ M}^{-1} \text{ s}^{-1}$.¹¹

Scheme 1.4 Dimerization step

Figure 1.2 Cyclic voltammograms of 4.14 mM 2,6-di-tert-butyl-4-ethylphenoxide in acetonitrile with 0.10 M tetraethylammonium perchlorate, glassy carbon electrode¹⁰

Adapted from *Acc. Chem. Res.* **1977**, 10, 313-319

Scheme 1.4 shows the reversible dimerization of 2, 6-di-tert-butyl-4-ethylphenoxy radical, and Figure 1.2 shows cyclic voltammograms of 2, 6-di-tert-butyl-4-ethylphenoxide in acetonitrile solvent. At slow scan rate (0.1 V s^{-1}), an obvious reductive peak appears at -0.3 V . It is caused by reduction of the phenoxy radical. At this scan rate, there is enough time to dissociate the quinol ether to phenoxy radical which is reduced back to phenoxide at -0.3 V . But at moderate scan rate (20 V s^{-1}), the reductive peak for the phenoxy radical disappears at -0.3 V . Instead a reductive peak is observed at -2.0 V which is caused by reduction of quinol ether. It is explained that the dimerization is so rapid that phenoxy radical reacts to form quinol ether which cannot undergo back-reaction. At slower scan rate (2 V s^{-1}), a small reductive peak appears at -0.35 V which is associated with another small reductive peak at -2.0 V . This is because not all the phenol radical forms quinol ether at this scan rate. Some phenol radical reduces back to phenoxide at -0.3 V . And the rest forms the quinol ether which is reduced at -2.0 V . So there are two reductive peaks at scan rate of 2.0 V s^{-1} .

1.2 Vitamin E

1.2.1 Properties and Functions of vitamin E

Many organic materials and food rapidly become rancid and deteriorate even when refrigerated, caused by a chemical reaction with atmosphere oxygen. If the reaction is under mild conditions, it is called autoxidation; and if the reaction occurs in biological circles, it is named lipid peroxidation. The process is represented in Equations 1.3-1.5.¹²

RH represents a lipid molecule and $R^{\bullet}$ the radical formed from R by removal of a hydrogen atom. First, $R^{\bullet}$ can be produced by heat, light or reaction with a redox agent. Then $R^{\bullet}$ reacts with oxygen quickly to form a peroxy radical $ROO^{\bullet}$ which can react with RH slowly to form ROOH and form a new $R^{\bullet}$. Equations 1.3 and 1.4 are termed propagation processes, which can be terminated by two chain carrying $ROO^{\bullet}$ radicals reacting together to form nonradical products.

When aerobic organisms are exposed to too much oxidative stress, polyunsaturated fatty acids which compose biomembranes and lipoproteins are easiest to undergo autoxidation. When autoxidation occurs, it has disastrous consequences for the organism. However, organisms that are alive do not turn rancid, because some materials are believed to prevent autoxidation process in our body. Such compounds are called antioxidants.¹³ Vitamin E is commonly known as a natural radical scavenging antioxidant. It is localized in the lipophilic cell membranes where there are a large amount of polyunsaturated fatty acids.¹⁴

Vitamin E was discovered by Evans et al. in 1922 as a necessary dietary compound essential for reproduction of rats.¹⁵ Later research showed feeding the rats and chickens with rancid fat resulted in various pathologies in these animals, which could be cured by ingesting wheat germ oil concentrates.¹⁶ So vitamin E was first characterised in wheat

germ oil and lettuce. Further investigation showed vitamin E includes a series of similar substances.¹⁷ The term ‘tocopherol’ was first suggested by Evans et al. in 1936. It is based on 6-chromanol with an extended saturated alkyl (phytyl) chain in the 2-position. Another series of substances containing three double bonds in the 3', 7' and 11' positions of the alkyl side chain (Scheme 1.5) were revealed and named ‘tocotrienols’ in 1964.^{18, 19} The α -, β -, γ - and δ -forms of the tocopherols and tocotrienols differ in the degree of methylation of the aromatic ring.

Tocopherol/tocotrienol	R ₁	R ₂	R ₃
α -TOH	CH ₃	CH ₃	CH ₃
β -TOH	CH ₃	H	CH ₃
γ -TOH	H	CH ₃	CH ₃
δ -TOH	H	H	CH ₃

Scheme 1.5 Structures of the vitamin E

There are currently controversies on the biological function of vitamin E. Most researchers including Traber et al. take the classical view that vitamin E only acts as an antioxidant in biomembranes.²⁰ In contrast Azzi has stated “ α -tocopherol is not able, at physiological concentrations to protect against oxidant-induced damage or prevent disease allegedly caused by oxidative damage”.²¹ Although they have different opinions, both of them agree that tocopherols can act as antioxidants in vivo, even in pharmacological doses. The key point of their controversy is whether pharmacological doses are physiologically or pathologically relevant,²² meaning that even if the antioxidant mechanism can occur in vivo, it does not mean that it is biologically important.

Since it is widely accepted that vitamin E acts as an antioxidant in vivo, it is necessary to discuss the mechanism of how to terminate the autoxidation process. The scavenging role is demonstrated in Equation 1.6-1.7. First, vitamin E reacts with an oxidised species in a lipophilic domain ($\text{ROO}^\bullet$) to form ROOH and the tocopheroxyl radical ($\text{TO}^\bullet$) (Equation 1.6).

Then the $\text{TO}^\bullet$ radical reacts with another $\text{ROO}^\bullet$ to yield a molecule of $(\text{TO})\text{OOR}$ (Equation 1.7). In this mechanism, one TOH is able to scavenge two $\text{ROO}^\bullet$ radicals.²³

It has been proposed that vitamin E has more functions than being an antioxidant in biomembranes, such as stabilizing membranes, inhibiting platelet aggregation and maintaining the immune system.²⁴ Investigations have shown that α -tocopherol has some

functions which are not shared with other forms of vitamin E. For example, protein kinases C (also known as PKC) are a family of enzymes that regulate biological processes such as cell growth. Because they have a great effect on a cell, their activity is highly regulated. Boscoboinik et al.²⁵ and Tasinato et al.²⁶ found that α -tocopherol is able to inhibit smooth muscle cell growth and decrease protein kinases C activity. But these functions are not shared with the β -, γ - and δ -tocopherols. β -tocopherol does not effectively inhibit PKC, however, it can prevent the inhibitory consequence of α -tocopherol. And γ - and δ -tocopherols do not perform any of these functions.²⁷ Moreover, α -tocopherol is able to modulate a number of cell functions which include preservation of endothelial integrity and inhibition of monocyte-endothelial processes.^{25,28} These modulation functions are not shared by the other forms of vitamin E.

1.2.2 Location of vitamin E

The way in which tocopherols are organized and distributed within the membranes is thought to be important in controlling the molecular mechanisms.¹⁷ There are plentiful studies on how vitamin E is located in biological membranes. Certain phospholipid classes dispersed in aqueous media give rise to assembly of the molecules into bilayers spontaneously. This kind of bilayer is always used as a model for the lipid template of membranes. Niki et al.²⁹ showed the phytol chain of α -tocopherol attaches the molecule tightly within the phospholipid bilayer by ESR probe experiments.

There are three different proposed locations and orientation of α -tocopherol within phospholipid bilayer membranes.³⁰ Fragata et al.³¹ and Lessard et al.³² suggested that the OH-group of α -tocopherol forms a hydrogen bond with the ester carbonyl group of the lipid within about 1 nm within the membranes. In contrary, Srivastava et al.³³ and Perly et

al.³⁴ proposed that the OH-group of α -tocopherol forms a hydrogen bond with the phosphate group of the phospholipid bilayer based on ^{13}C -NMR experiments. These two suggestions indicated that the chromanol ring of α -tocopherol is oriented to the lipid-water interface of the phospholipid bilayer, but does not reach out to the lipid-water interface. The third proposal is reported by Bisby et al.³⁵ They showed the chromanol ring of α -tocopherol is located in different regions of the lipid bilayer when they are in different phase. For example, it is close to the surface of a lipid bilayer when it is in the gel phase; however, it is located in the hydrophobic inner domain of the lipid bilayer when it is in the fluid phase.

1.2.3 Electrochemistry of vitamin E

1.2.3.1 Electrochemistry of α -tocopherol

α -Tocopherol can be oxidised by one-electron to form the cation radical, $\alpha\text{-TOH}^{\bullet+}$ (Equation 1.8) at approximately +0.5 V vs Fc/Fc^+ in acetonitrile without added acid or base.²³ $\alpha\text{-TOH}^{\bullet+}$ quickly loses a proton to form $\alpha\text{-TO}^\bullet$ (Equation 1.9). $\alpha\text{-TO}^\bullet$ is immediately further oxidised by one electron to form the phenoxonium cation, $\alpha\text{-TO}^+$ (Equation 1.10). The mechanism can be termed as ECE process (Figure 1.3, green line).⁸

³⁶ The phenoxonium cation, $\alpha\text{-TO}^+$ is stable for several hours in CH_3CN without water and it is able to be converted back to $\alpha\text{-TOH}$ by applying a reducing potential.

Figure 1.3 Cyclic voltammograms of 2mM substrates at 1mm diameter Pt electrode in CH_3CN with 0.25M Bu_4NPF_6 : red line--- α -TOH with 0.1 M $\text{CF}_3\text{SO}_3\text{H}$; blue line--- α -TOH reacted with 2 mM Et_4NOH to form $\alpha\text{-TO}^-$; green line--- α -TOH, scan rate: 0.1 V s^{-1} .⁸ Adapted from *J. Am. Chem. Soc.* **2004**, 126, 12441-12450

When $\text{CF}_3\text{SO}_3\text{H}$ acid is added to solutions of α -TOH in CH_3CN , Equation 1.9 favours the protonation reaction and $\alpha\text{-TOH}^{+\bullet}$ has an increased lifetime. At very high acid concentrations, α -TOH is able to be oxidised by one-electron to form $\alpha\text{-TOH}^{+\bullet}$ at $\sim +0.6 \text{ V}$ vs Fc/Fc^+ . 0.1M $\text{CF}_3\text{SO}_3\text{H}$ in CH_3CN is high enough to stop the deprotonation process and stabilize $\alpha\text{-TOH}^{+\bullet}$. At $\sim +1.4 \text{ V}$ vs Fc/Fc^+ , $\alpha\text{-TOH}^{+\bullet}$ can be further oxidised by one electron to form $\alpha\text{-TOH}^{2+}$ (Equation 1.11). The cyclic voltammogram is shown in Figure 1.3 (red line). The dication is not stable for more than a few seconds at most according to electrolysis and spectroelectrochemistry results.³⁶ It loses a proton quickly to form $\alpha\text{-TO}^+$ (Equation 1.12).

$\alpha\text{-TO}^+$ is stable in acid conditions and is able to reduce back to $\alpha\text{-TOH}$ by two one-electron, one proton steps.³⁷ So, in an acid environment, the mechanism for the forward oxidation reaction is different from the backward reduction reaction.

When an organosoluble base, like Et_4NOH is added to $\alpha\text{-TOH}$ in CH_3CN , it forms the phenolate anion, $\alpha\text{-TO}^-$ quickly.^{8, 38, 39} The anion can be oxidised to form $\alpha\text{-TO}^+$ by two electrons (Equation 1.13-1.15). $\alpha\text{-TO}^-$ is oxidised at -0.9 V vs Fc/Fc^+ , which means that it is easier to oxidise than $\alpha\text{-TOH}$. Therefore, in Figure 1.3 (blue line), the potential is substantially shifted to a more negative position. The behaviour of $\alpha\text{-TOH}$ in basic conditions is typical of most phenols. They easily lose a proton to form the phenolate anions and can be oxidised.^{4, 8, 38-40}

The overall mechanism is shown in Scheme 1.6,^{8, 23, 36, 39, 41-43} which has been proven by slow scan rate CV and controlled potential electrolysis experiments. Detailed spectroscopic analysis such as EPR, FTIR, NMR and UV-vis spectroscopies have also been used to determine the overall mechanism and identify intermediate species.

^aOne resonance structure is displayed for each compound. The listed potentials (vs. ferrocene^{0/+}) were obtained by voltammetry and are the approximate values necessary to bring about oxidation of the phenolic compounds but do not necessarily correspond to the formal potential. The counter ions for the charged species are the supporting electrolyte cation [Bu₄N⁺] and anion [PF₆⁻], and the "H⁺" ions exist coordinated to the organic solvent. R₁, R₂, R₃=H or CH₃. R₄=CH₃ or (CH₂CH₂CH₂CH(CH₃))₃CH₃).

Scheme 1.6 Electrochemically induced transformations of vitamin E and model compounds in dry CH₃CN or CH₂Cl₂^{8, 23, 36, 39, 41-43 a} Adapted from *J. Am. Chem. Soc.* **2004**, 126, 12441-12450

1.2.3.2 Electrochemistry of β -, γ - and δ -tocopherols

β -, γ - and δ -tocopherols undergo the same mechanism as α -TOH.³⁶ However, the stability of their phenoxonium cation are different. α -TO⁺ is able to last several hours in dry organic solvents (CH₃CN and CH₂Cl₂). But β -TO⁺ is only stable for several minutes and γ - and δ -TO⁺ are stable for less than one second. It is still unknown why α -TO⁺ and β -TO⁺ can last longer in solution than the other two cations. Both of them have a methyl group in R₁ (Scheme 1. 5), and can react to form the *o*-quinone methide. It is also possible that the phenolic methyl groups can inhibit reactions with nucleophiles by stabilizing the phenoxonium ions sterically.

1.2.4 Spectroscopic properties of oxidised forms of all tocopherols

Large amounts of spectroscopic data on different oxidised forms of all the tocopherols except the dications in Equations 1.8-1.15 have been collected.^{8, 36, 37} The infrared spectra of α -TO⁺ which is formed by electrochemical oxidation of α -TOH or chemical reaction with NO⁺ showed three strong bands at 1605, 1650 and 1670 cm⁻¹.^{8, 36, 41} These bands have been assigned to the asymmetric C=C ring stretch, symmetric C=C ring stretch and C=O stretch.⁴¹ The radical, α -TO[•], has been identified by electron paramagnetic resonance (EPR) spectroscopy.^{39, 44-46} EPR spectra of the cation radicals of all the tocopherols which were obtained by chemical oxidation have been characterized in CH₂Cl₂ at low temperatures.^{47, 48} UV-vis spectra of the cation radicals of all the tocopherols in CF₃COOH or CH₃CN with CF₃SO₃H have also been measured.^{8, 36, 37} They showed two bands at 22000 and 33000 cm⁻¹, which were also obtained in the UV-vis spectrum of α - and β -TO⁺. However, the intensities and shapes of the UV-vis bands of the cation radicals

and cations were different. The UV-vis spectrum of α -TOH⁺ was reported in hexane by pulse radiolysis experiments.⁴⁹

1.3 Electrochemistry of lipid layers

The electrochemistry of vitamin E is often studied in organic solvents due to the solubility properties.^{8, 23} However, vitamin E exists naturally in lipid bilayer membranes. The study of small molecules like α -TOH in biological systems is complicated because it is necessary to transfer a suitable counterion to the system during the charge transfer processes in order for current to flow. However, for high molecular weight biological molecules like proteins and enzymes, it is easy to balance the charge brought about by the electron transfer reactions. Because the bulk of the macromolecule lies outside of the surface confined layers and ions from solutions are easy to transfer across the membrane-aqueous solution interface. The discussion below summarizes some of the methods that have been used to study electron transfer reactions of molecules through surface modified electrodes.

1.3.1 Self-assembled monolayers (SAMs)

Self-assembled monolayers (SAMs) on solid support have been discussed for many years.⁵⁰ Scheme 1.7 shows the metal modified with an “X-Y” SAM. X is usually thiolate which is bound to the metal surface, and Y is a functional group which can react with solution and protein molecules. Y can be changed according to the different properties of protein.

Scheme 1.7 Metal modified with an “X-Y” SAM⁵¹ Adapted from *Electrochim. Acta* **2000**, 45, 2623-2645

“X-Y” is usually the promoter for the electron transfer between the electrode and proteins in solution. Taniguchi et al.⁵² used a bis(4-pyridyl) disulfide modified gold electrode to study the electrochemistry of proteins (cytochrome c) for the first time. They found the rate of the electron transfer between cytochrome c and gold surface was increased when the electrode was modified with bis(4-pyridyl) disulfide. It was interesting to find using mixed SAM which comprised active “X-Y” adsorbates and non-functionalized (“X-Y”) n-alkanethiol blocked the protein interaction. Sato et al.⁵³ applied SAM containing a mixture of bis(4-pyridyl) disulfide and n-alkanethiol with various chain lengths on gold electrode to investigate the electrochemical response of cytochrome c. Interestingly, the mixed monolayers with shorter alkanethiol chains block the electron transfer more effectively than those with longer chains. Scheme 1.8⁵³ is the proposed model for the mixed SAM with longer chain n-alkanethiol and cytochrome c. It is proposed that the interaction of bis(4-pyridyl) disulfide and n-alkanethiol with longer

chains is so weak, leaving enough area for the interaction of bis(4-pyridyl) disulfide and cytochrome c.⁵¹

Scheme 1.8 Proposed structure of the mixed SAM [bis(4-pyridyl) disulfide and n-alkanethiol with longer chain] and cytochrome c⁵³ Adapted from *J.Electroanal. Chem.* **1997**, 438, 99-104

1.3.2 Supported lipid membranes

Proteins also can be immobilized in surfactant films which form lipid bilayers and multilayers. Surfactants are usually organic compounds which are amphiphilic. They contain hydrophobic tails which are usually hydrocarbon chains and have hydrophilic heads.⁵⁴ Suitable surfactants for stable films are those which are insoluble in water and do not form micelles.⁵⁴ Some surfactants are shown in Scheme 1.9. Phosphatidylcholines are a class of phospholipid and are major components of biological membranes.

DDAB-didodecyldimethylammonium bromide

DHP- dihexadecylphosphate

DMPC- dimyristoylphosphatidylcholine

Scheme 1.9 Structures of some surfactants

Surfactants can be deposited on electrode surfaces by simple procedures. Evaporation of organic solvents which dissolve the surfactant leaves self-assembled bilayers or multilayers. There are several methods to embed protein into films. In the first, the method is similar to cast film on the electrode surface. The surfactant-coated electrode is prepared and placed into a solution containing a protein which diffuses into the surfactant film. Another way is to prepare a mixed aqueous vesicle dispersion containing surfactant and protein. Then the mixture is spread onto an electrode surface and dried.⁵⁵ These procedures result in forming multilayers as in Scheme 1.10. Most films which comprise surfactant and protein can be deposited on PG (pyrolytic graphite), Au and Pt electrode surfaces. And they can last for one month in buffer.^{54, 55}

Scheme 1.10 An electrode modified with surfactant multilayer containing protein molecules⁵¹ Adapted from *Electrochim. Acta* **2000**, 45, 2623-2645

For example, voltammetry of myoglobin (Mb) which is an oxygen transport protein in mammalian muscle shows slow electron transfer at bare electrode surface.⁵⁴ But the electron transfer rates are enhanced with incorporation of Mb in the films of DDAB, DMPC and other surfactants on Pt, Au or Pt electrode surface.⁵⁵⁻⁵⁹

1.3.3 Hybrid membranes

Hawkrige et al.⁶⁰ reported so called hybrid bilayers in which a SAM is formed on gold or silver electrodes, followed by addition of a phospholipid monolayer on the submonolayer (Scheme 1.11). The sub-SAM is typically from an alkanethiol and the phospholipid monolayers are also formed by self-assembly. Numerous experiments using thiol self-assembly methods showed that alkanethiol monolayers on gold can provide a

very stable membrane assembly because the thiols react with gold strongly by covalent linkage and form a well-defined structure.⁶⁰ This kind of hybrid bilayers have advantages

Scheme 1.11 A hybrid bilayer membrane consists of a partial self assembled monolayer of the octadecyl mercaptan (OM) and a phospholipid monolayer⁶⁰ Adapted from *Langmiur* **1994**, 10, 877-882

of reproducibility and long-term stability.⁶¹ Hawkrigde et al.⁶⁰ incorporated cytochrome c oxidase into the hybrid bilayer membranes attached to gold surfaces. First, a stable submonolayer of octadecyl mercaptan (OM) was formed on a gold electrode. Appropriate concentration of OM and modification time of forming the submonolayer can make the electrode surface partially covered with OM. But for this procedure the reproducibility is hard to control. Then the electrode is placed into a dialysis tube which contains deoxycholate, lipid buffer and cytochrome c oxidase. After the deoxycholate dialyzes out, the enzyme has been incorporated into the lipid-layer with a controlled orientation. The

voltammetric data shows direct electron transfer between the gold electrode and cytochrome c oxidase.

1.3.4 Tethered lipid membranes

Hybrid membranes are not suitable for incorporating integral proteins because the structure of the hybrid bilayer contains a high proportion of hydrophobic parts and is too rigid.⁶² Lack of flexibility and fluidity makes the hybrid bilayer difficult for ion transport. To overcome these drawbacks, the alkanethiol monolayer has been replaced by thiolipid monolayer. The membranes are “tethered” to the surface. Thiolipid is a class of lipid which consists of a hydrophilic spacer associated with a sulphhydryl or disulfide group at one end and the polar head of phospholipid at the other end.⁶³ Scheme 1.12 is the example of chemical structure of thiolipids.⁶² The polyethyleneoxy chain is the hydrophilic spacer.

Scheme 1.12 Chemical structure of thiolipids⁶² Adapted from *Langmiur* **1994**, 10, 197-210

“Tethered” membrane is intermediate to the hybrid and supported lipid bilayer. Devadoss et al.⁶⁴ have bound thiol-functionalized lipid monolayers on ITO surfaces. This monolayer is as the inner lipid leaflet of bilayers. Then by using a deoxycholate dialysis procedure, the outer lipid leaflet immobilization of cholesterol oxidase is deposited on the inner lipid monolayer (Scheme 1.13). Amperometric responses for the cholesterol oxidase in the presence of cholesterol solutions show cholesterol partitions into the lipid bilayer membranes from solution as the hydrophobic interaction between the lipid tails and cholesterol. Cholesterol readily produces hydrogen peroxide which is electrochemically reduced at the electrode surface (Equation 1.16 and 1.17).

Scheme 1.13 Idealized “tethered” bilayer containing cholesterol oxidase⁶⁴ Adapted from *Langmiur* **2002**, 18, 9617-9621

Membrane modified electrodes provide a promising opportunity to study large molecules like proteins and enzymes in their native environment. But there are some limitations. Because the lipid membranes reduce the mobility of large molecules on the electrode surface, the electron transfer rate is limited. By controlling the orientation of the large molecules on the electrode surface or addition of mediators, it might improve the electron transfer rate.^{65, 66}

1.4 Aim of the present project

There are numerous electrochemical experiments of vitamin E performed in organic solvents to date.^{23, 39-41} In this thesis, a model membrane environment was developed to enable voltammetric experiments to be performed on biological molecules incorporated inside artificial lipid bilayer (or multilayer) membranes. Lecithin was chosen for lipid bilayers, because it is a naturally occurring phospholipid present in plant and animal tissues. The artificial supported membranes were prepared by incorporating vitamin E into lecithin multilayers or sandwiching multilayers of lecithin between layers of Nafion that were deposited on the surface of a glassy carbon electrode. Cyclic voltammetric behaviour was examined at different scan rates and different pH buffered aqueous solutions.

References:

1. Lund, H.; Hammerich, O., *Organic Electrochemistry 4th ed.* Marcel Dekker: New York, 2001.
2. Rieker, A.; Beisswenger, R.; Regier, K., *Tetrahedron* **1991**, 47, 645-654.
3. Speiser, B.; Rieker, A., *J. Electroanal. Chem.* **1979**, 102, 373-395.
4. Evans, D. H.; Jimenez, P. J.; Kelly, M. J., *J. Electroanal. Chem.* **1984**, 163, 145-157.
5. Richards, J. A.; Evans, D. H., *J. Electroanal. Chem.* **1977**, 81, 171-187.
6. Richards, J. A.; Whitson, P. E.; Evans, D. H., *J. Electroanal. Chem.* **1975**, 63, 311-327.
7. Hapiot, P.; Pinson, J., *J. Electroanal. Chem.* **1993**, 362, 257-265.
8. Williams, L. L.; Webster, R. D., *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.
9. Eickhoff, H.; Jung, G.; Rieker, A., *Tetrahedron* **2001**, 57, 353-364.
10. Evans, D. H., *Acc. Chem. Res.* **1977**, 10, 313-319.
11. Land, E. J.; Porter, G., *Trans. Faraday Soc.* **1963**, 59, 2016-2026.
12. Burton, G. W.; Ingold, K. U., *Acc. Chem. Res.* **1986**, 19, 194-201.
13. Ingold, K. U., *Chem. Rev.* **1961**, 61, 563-589.
14. Niki, E.; Noguchi, N., *Acc. Chem. Res.* **2004**, 37, 45-51.
15. Evans, H. M.; Bishop, K. S., *Science* **1922**, 56, 650-651.
16. Wolf, G., *Mattill. J. Nutr.* **2005**, 135, 363-366.
17. Wang, X.; Quinn, P. J., *Prog. Lipid Res.* **1999**, 38, 309-336.
18. Pennock, J. F.; Hemming, F. M.; Kerr, J. D., *Biochem. Biophys. Res. Commun.* **1964**, 17, 542-549

19. Whittle, K. J.; Dunphy, P. J.; Pennock, J. F., *J. Biochem.* **1966**, 100, 138-143.
20. Traber, M. G.; Atkinson, J., *Free Radical Biol. Med.* **2007**, 43, 4-15.
21. Azzi, A., *Free Radical Biol. Med.* **2007**, 43, 16-21.
22. Brigelius-Flohe, R.; Davies, K. J. A., *Free Radical Biol. Med.* **2007**, 43, 2-3.
23. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
24. Aranda, F. J.; Sanchez-Migallon, M. P.; Gamez-Fernandez, J. C., *Archives of Biochemistry And Biophysics* **1996**, 333, 394-400.
25. Boscoboinik, D.; Szewczyk, A.; Hensey, C.; Azzi, A., *J. Biol. Chem.* **1991**, 266, 6188-6194.
26. Tasinato, A.; Boscoboinik, D.; Bartoli, G. M.; Maroni, P.; Azzi, A., *Proc. Natl. Acad. Sci. U. S. A.* **1995**, 92, 12190-12194.
27. Ricciarelli, R.; Zingg, J.-M.; Azzi, A., *Biol. Chem* **2002**, 383, 457-465.
28. Mahoney, C. W.; Azzi, A., *Biochem. Biophys. Res. Commun.* **1988**, 154, 694-697.
29. Niki, E.; Kawakami, A.; Saito, M.; Yamamoto, Y.; Tsuchiya, J.; Kamiya, Y., *J. Biol. Chem.* **1985**, 260, 2191-2196.
30. Fukuzawa, K.; Ikebata, W.; Shibata, A.; Kumadaki, I.; Sakanaka, T.; Urano, S., *Chem. Phys. Lipid* **1992**, 63, 69-75.
31. Fragata, M.; Bellemare, F., *Chem. Phys. Lipid* **1980**, 27, 93-99.
32. Lessard, J. G.; Fragata, M., *J. Phys. Chem* **1986**, 90, 811-817.
33. Srivastava, S.; Phadke, R. S.; Govil, G.; Rao, C. N. R., *Biochim. Biophys. Acta* **1983**, 734, 353-362.
34. Perly, B.; Smith, I. C. P.; Hughes, L.; Burton, G. W.; Ingold, K. U., *Biochim. Biophys. Acta* **1985**, 819, 131-135.

35. Bisby, R. H.; Ahmed, S., *Free Radical Biol. Med.* **1989**, 6, 231-239.
36. Wilson, G. J.; Lin, C. Y.; Webster, R. D., *J. Phys. Chem. B* **2006**, 110, 11540-11548.
37. Svanholm, U.; Bechgaard, K.; Parker, V. D., *J. Am. Chem. Soc.* **1974**, 96, 2409-2413.
38. Nanni, E. J.; Jr.; Stallings, M. D.; Sawyer, D. T., *J. Am. Chem. Soc.* **1980**, 102, 4481-4485.
39. Webster, R. D., *Electrochem. Commun.* **1999**, 1, 581-584.
40. Webster, R. D., *Electrochem. Commun.* **2003**, 5, 6-11.
41. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D., *J.Org.Chem.* **2005**, 70, 10466-10473.
42. Lee, S. B.; Willis, A. C.; Webster, R. D., *J. Am. Chem. Soc.* **2006**, 128, 9332-9333.
43. Peng, H. M.; Webster, R. D., *J. Org. Chem.* **2008**, 73, 2169-2175.
44. Bowry, V. W.; Ingold, K. U., *J. Org. Chem.* **1995**, 60, 5456-5467.
45. Mukai, K.; Tsuzuki, N.; Ishizu, K.; Ouchi, S.; Fukuzawa, K., *Chem. Phys. Lipid* **1981**, 29, 129-135.
46. Matsuo, M.; Matsumoto, S.; Ozawa, T., *Org. Magn. Reson* **1983**, 21, 261-264.
47. Mukai, K.; Tsuzuki, N.; Ouchi, S.; Fukuzawa, K., *Chem. Phys. Lipid* **1984**, 35, 1928-1933.
48. Lehtovuori, P.; Joela, H., *Phys. Chem. Chem. Phys.* **2002**, 4, 1928-1933.
49. Edge, R.; Land, E. J.; McGarvey, D.; Mulroy, L.; Truscott, T. G., *J. Am. Chem. Soc.* **1998**, 120, 4087-4090.

50. Pierrat, O.; Lechat, N.; Bourdillon, C.; Laval, J.-M., *Langmiur* **1997**, 13, 4112-4118.
51. Armstrong, F. A.; Wilson, G. S., *Electrochim. Acta* **2000**, 45, 2623-2645.
52. Taniguchi, I.; Toyosawa, K.; Yamaguchi, H.; Yasukouchi, K., *J. Chem. Soc. Chem. Commun.* **1982**, 1032-1033.
53. Sato, Y.; Mizutani, F., *J. Electroanal. Chem.* **1997**, 438, 99-104.
54. Rusling, J. F., *Acc. Chem. Res.* **1998**, 31, 363-369.
55. Nassar, A.-E. F.; Willis, W. S.; Rusling, J. F., *Anal. Chem.* **1995**, 67, 2386-2392.
56. Nassar, A.-E. F.; Narikiyo, Y.; Sagara, T.; Nakashima, N.; Rusling, J. F., *J. Chem. Soc. Faraday Trans.* **1995**, 91, 1775-1782.
57. Nassar, A.-E. F.; Zhang, Z.; Hu, N.; Rusling, J. F.; Kumosinski, T. F., *J. Phys. Chem. B* **1997**, 101, 2224-2231.
58. Rusling, J. F.; Nassar, A.-E. F., *J. Am. Chem. Soc.* **1993**, 115, 11891-11897.
59. Zhang, Z.; Rusling, J. F., *Biophys. Chem.* **1997**, 63, 133-146.
60. Cullison, J. K.; Hawkrige, F. M.; Nakashima, N.; Yoshikawa, S., *Langmiur* **1994**, 10, 877-882.
61. Plant, A. L., *Langmiur* **1993**, 9, 2764-2767.
62. Lang, H.; Duschl, C.; Vogel, H., *Langmiur* **1994**, 10, 197-210.
63. Guidelli, R.; Aloisi, G.; Becucci, L.; Dolfi, A.; Moncelli, M. R.; Buoninsegni, F. T., *J. Electroanal. Chem.* **2001**, 504, 1-28.
64. Devadoss, A.; Burgess, J. D., *Langmiur* **2002**, 18, 9617-9621.
65. Marchal, D.; Pantigny, J.; Laval, J. M.; Moiroux, J.; Bourdillon, C., *Biochemistry* **2001**, 40, 1248-1256.

66. Weiss, S. A.; Bushby, R. J.; Evans, S. D.; Henderson, P. J. F.; Jeuken, L. J. C.,
Biochem. J. **2009**, 417, 555-560.

Chapter 2

Experimental Section

2.1 Theory of cyclic voltammetry

Cyclic voltammetry (CV) is a widely used technique to explore the mechanism of electrochemical oxidations or reductions and the chemical reversibility of processes which take place at the electrode surface.

2.1.1 A chemically reversible process

Consider a simple one electron-transfer process as in Equation 2.1.

Figure 2.1 Typical cyclic voltammogram for one electron transfer process

The shape of a typical one-electron chemically reversible cyclic voltammogram is shown in Figure 2.1. The current begins to rise at point (a) due to the oxidation of A at the working electrode. Scanning from point (a) to a more positive potential, the concentration of starting material (A) at the electrode surface ($[A]_{x=0}$) decreases because A is oxidized to B. Thus, there is a steady increase in current. As $[A]_{x=0}$ approaches zero, the current reaches its peak. However at this time, starting material (A) must diffuse through the solution because the diffusion layer tries to equalize the concentration of A and B. Then scanning to more positive value, the current steadily decreases. This drop in current is called the “diffusion limited current”.¹ And the peak shows the typical asymmetric shape. At point (b) the potential is reversed; this point in the voltammogram is termed the “switching potential”. At first, the concentration of B is very high at the electrode surface. On scanning the potential from point (b) to a more negative value, B is reduced back to A. And the same asymmetric shape is observed on the reverse scan. When the peak current I_p^{ox} is equal to $-I_p^{red}$, the process is deemed to be chemically reversible. However, when $(-I_p^{ox}/I_p^{red})$ is more than or less than 1, it will not be a chemically reversible reaction and hence it is possible to determine other information such as the kinetic processes. If the diffusion coefficients of A and B are equal, the midpoint E_m (Equation 2.2) or half potential $E_{1/2}^r$ equals the formal potential $E_f^\ominus$.

$$E_m = E_{1/2}^r = \frac{E_p^{ox} + E_p^{red}}{2} \quad (2.2)$$

2.1.2 A chemically irreversible process

Consider a chemical reaction coupled to the electron-transfer process. The EC (“E” represents electron transfer process and “C” represents a chemical step) mechanism is illustrated in Equations 2.3-2.4.

Figure 2.2 Simulated cyclic voltammogram for an EC mechanism

Species A is oxidized to B and B undergoes a chemical step to form species C. If the electron-transfer step is chemically reversible and $k_b \gg k_f$, the chemical reaction is outrun at faster scan rates. The peak shape is shown in Figure 2.2a, which is just like a simple one electron-transfer step; if $k_f \gg k_b$ for the C step, then species A can be converted to C.

Therefore, the reversible peak may disappear. However, for a less fast chemical reaction, a reductive peak still appears. But it will be reduced in magnitude as in Figure 2.2b.

2.2 Electrochemical cell for cyclic voltammetry

Scheme 2.1 Typical electrochemical cell for cyclic voltammetry

There are three electrodes (working, reference and counter) included in the cell which is shown in Scheme 2.1. The working electrode is usually placed in the centre. The reference electrode is close to working electrode in order to minimize the IR drop (I equals current, R equals uncompensated resistance). Before doing the experiment, inert gas (nitrogen or argon) is introduced into the cell to remove the oxygen from the solution. Argon is preferred because it is heavier than air and better for an air-sensitive system. Supporting electrolyte is added to the solution in most voltammetric experiments. It consists of an anion and a cation which are not easily oxidized and reduced, such as $\text{Bu}_4\text{N}^+\text{PF}_6^-$. The effect of the supporting electrolyte is to minimize the solution resistance, reduce migration effects and provide a means of passing current throughout the solution. In the case of ionic liquid, the solvent can act as the electrolyte.

The working electrode is made from electrically conducting material. Normally it includes solid platinum, gold, silver and conducting forms of carbon, like glassy carbon and graphite. In earlier times, mercury electrodes² were often used for studying electrochemical processes. But mercury and related compounds are toxic pollutants which are harmful to the environment. Hence, their use should be controlled and limited. The electrodes can be classified as macro- and microelectrodes depending on their sizes. The diameter of macroelectrode is in mm range, whereas the diameter of microelectrodes is in the nm to μm range.

Reference electrodes have a stable and well-known potential. In aqueous solution, widely used reference electrodes are the silver/silver chloride (Ag/AgCl) electrode and calomel reference electrodes. The silver/silver chloride reference electrode consists of a silver wire which is coated with a silver chloride layer. Then the silver wire is dipped into a KCl solution with a concentration from 1.0 M to saturated. A porous plug is on the end. In order to avoid the contamination of the KCl solution, salt bridge is present outside the Ag/AgCl electrode. However, if aqueous Ag/AgCl electrode is used in organic solvent, it can cause liquid junction potential and alter the cell potential. The liquid junction potential at the reference-salt bridge and salt bridge-organic solvent interfaces will alter the cell potential.³

Hence in organic solvents, it is common to use ferrocene (Fc) as the reference scale. The mechanism of oxidation of ferrocene is shown in Equation 2.5. This process has been proposed as standard one electron-transfer reaction⁴ and exhibits reversible behaviour. Care must be taken to avoid the potential of Fc/Fc^+ so that it does not overlap with the

process of interest. And the oxidised and reduced species being studied do not react with Fc and Fc⁺ forms.

The counter electrode is also called the auxiliary electrode. It is often fabricated from inert material, like platinum or carbon. The current flows through the working and counter electrode. If an oxidative process occurs at the working electrode, a reductive reaction must occur at the counter electrode.

2.3 Microelectrodes

Microelectrodes are described as having at least one electrode dimension with range from nm to tens of μm . There are a lot of geometries of microelectrode with the most important one being the microdisc electrode. Usually it is made by inserting a sufficiently small diameter wire into an insulating material, such as resin.⁵ Compared to macroelectrodes, this kind of small sized electrode has the following attractive properties: a) the ohmic drop of potential, IR , is decreased because the current is very low; b) steady state condition is attained rapidly; c) small volume analytes are permitted; d) because of the suppressed charging current, the potential can be swept at a fast rate.⁶

Figure 2.3 provides an example of the effect of electrode radius on the ohmic drop in cyclic voltammograms.⁷ In this case, the solvent is chlorobenzene solution whereby its resistance is approximately 300 times greater than that of typical aqueous solutions. At the bigger electrode, the peak separation is increased and the peak potential is shifted by over 600 mV from its true position. This is caused by ohmic drop. At the same time, for the

larger electrode, the upper scan rate limit is $\sim 1 \text{ V s}^{-1}$ because of ohmic drop. However, this limit of scan rate can be increased by using microelectrodes.

Figure 2.3 Cyclic voltammograms of 1.1 mM ferrocene in chlorobenzene with 0.5 M tetra-n-hexylammonium perchlorate supporting electrolyte at 0.8 mm (solid line) and 52 μm (dotted line) radius gold disk electrodes. The scan rate = 50 V s^{-1} .⁷ Adapted from *Acc. Chem. Res.* **1990**, 23, 64-70

2.4 Rotating-disc electrode (RDE)

In a voltammetric experiment, there are three different processes by which the soluble species reach and are removed from the electrode. The first form is diffusion which is defined as the movement of species due to a concentration gradient. The second form is convection in which the movement is caused by the external forces. And the last mode is migration which is due to the movement of charged species, also a potential gradient.⁸ Scheme 2.2 is the representation of the three forms of the species to the electrode. All these movements are forms of mass transport.

Scheme 2.2 Three different forms of transport to the electrode

A rotating-disc electrode is a device in which the mass transfer of the species is mostly caused by the convection. This character permits the rotating-disc electrode to be used to calculate the relevant transport parameters, such as diffusion coefficient (D_i).⁹

The rotating- disk electrode is surrounded by a non-conducting sheath of substantially larger diameter.¹⁰ The whole electrode assembly is rotated about the axis perpendicular to the disc electrode. This rotation leads to a well-defined solution flow pattern as in Scheme 2.3.

Scheme 2.3 Solution movement associated with a rotating-disc electrode

Montiel et al.⁹ used an RDE of vitreous carbon to calculate the diffusion coefficient of Fe(CN)_6^{3-} and Fe(CN)_6^{4-} for a solution of $\text{K}_3\text{Fe(CN)}_6$ and $\text{K}_4\text{Fe(CN)}_6$ in Na_2SO_4 ; Abruna et al.¹¹ employed rotating disc electrode voltammetry to examine the number of electrons exchanged in BH_4^- oxidation steps and the heterogeneous charge transfer rate constant (k_0), and whether coupled chemical reactions occur.

2.5 Spectroscopic techniques

Several spectroscopic techniques have been combined with electrochemical methods to investigate the redox species formed.

2.5.1 Fourier transform infrared spectroscopy (FT-IR)

FT-IR is considered as one of the most powerful tools for identifying (fingerprinting) chemicals. The key part of the FT-IR is a Michelson interferometer which is shown in Scheme 2.4.^{12, 13} One arm of the interferometer includes a stationary mirror; the other arm includes a moving mirror. When the light is produced, it is split into two separate beams

by the beam splitter. These two beams are incident on the stationary mirror and moving mirror. Then they are both reflected back to the beam splitter and to the detector.¹⁴

Scheme 2.4 The Michelson interferometer^{12, 13} Adapted from Chemical Infrared Fourier Transform Spectroscopy. Wiley-Interscience: New York, **1975**

There are many ways to prepare the samples for FT-IR. For liquid samples, one drop of sample is usually placed between two plates of sodium chloride (NaCl salt). This salt is transparent to infrared light. For solid samples, they can be milled with potassium bromide (KBr) to form a fine powder which is then compressed into a thin film. KBr is also transparent to IR radiation. The other way to prepare the solid samples is dissolving the solid samples in a solvent. The mixture can be placed onto a salt plate. Then the solvent can be evaporated off to leave a thin film of original compound on the plate. For small samples, a microscope attachment can be connected to the FT-IR machine to obtain transmittance or reflectance spectra on small sized particles. Scheme 2.5 is the representation of FT-IR microscope.

Scheme 2.5 FT-IR microscope, the left is reflectance mode; the right is transmittance mode¹⁵ Adapted from www.thermo.com

Theoretically the FT-IR can obtain the spectrum from 200 to 4000 cm^{-1} , associated with the resolution of 1 cm^{-1} . Table 2.1 lists the characteristic infrared absorption peaks.¹⁶

For example, Hishikawa et al.¹⁷ used FT-IR combined with microscopic accessory to study the structure of crystalline cellulose. According to the characteristic IR bands for cellulose 1α and 1β crystalline phase during the formation of coniferous wood cell, the results show that the cellulose in the primary wall is rich in the 1α phase, however, 1β phase is rich in the secondary wall.

Table 2.1 Characteristic infrared absorption peaks¹⁶

	Functional group	Wavenumber/ cm^{-1}
O — H	Aliphatic and aromatic	3600-3000
NH ₂	Also secondary and tertiary	3600-3100
C — H	Aromatic	3150-3000
C — H	Aliphatic	3000-2850
C $\equiv$ N	Nitrile	2400-2200
C $\equiv$ C —	Alkyne	2260-2100
COOR	Ester	1750-1700
COOH	Carboxylic acid	1740-1670
C = O	Aldehydes and ketones	1740-1660
CONH ₂	Amides	1720-1640
C = C —	Alkene	1670-1610
R — O — R	Aliphatic	1160-1060

Zecchina et al.¹⁸ reported an in situ, time-resolved FT-IR spectroscopy at variable temperature of acetylene and methylacetylene oligomerisation on the $\text{Cr}^{2+}/\text{SiO}_2$ Phillips catalyst. The time-resolved FT-IR results show Cr sites can coordinate simultaneously three monomers and form benzene and 1, 3, 5-trimethylbenzene respectively.

2.5.2 UV/visible spectroscopy

UV/visible spectrometry is an essential tool in the identification and quantification of chemicals and biological substances.¹⁹ Scheme 2.6 shows the design of a single beam and

a double beam UV/visible spectrometer. The ultraviolet range is normally from 200 to 400 nm, and the visible region is from 400 to 800 nm.

(a)

(b)

Scheme 2.6 Diagram of UV/visible spectrometry (a) single beam instrument; (b) double beam instrument

For a double beam instrument, a beam of light from the light source is separated into its component wavelengths by the monochromator (or filter). Then this beam is split into two equal intensity beams by the beam splitter. One beam passes through the sample cell which contains a compound being studied in a solvent, and the other beam simultaneously passes through the reference cell which contains the solvent only. The intensities of these beams are measured by detector. The intensity of the reference beam is described as I_0 , and the intensity of reference beam is defined as I . Their ratio or the log of their ratio is obtained and displayed in the computer. The difference of the single beam instrument is

the monochromatic beam directly passes through reference cell. After removing the reference cell, the sample cell is measured.

Absorption is usually described as transmittance ($T = I/I_0$) or absorbance ($A = \log I_0/I$). The wavelength of maximum absorbance is defined as $\lambda_{\max}$. Different compounds may have different $\lambda_{\max}$ and absorbances. Table 2.2 is the absorption characteristics of some common organic chromophores.¹⁶

Table 2.2 Absorption characteristics of some common organic chromophores¹⁶

Chromophore	Example	Solvent	$\lambda_{\max}$, nm
Alkene	$\text{C}_6\text{H}_{13}\text{CH}=\text{CH}_2$	n-Heptane	177
Conjugated alkene	$\text{CH}_2=\text{CHCH}=\text{CH}_2$	n-Heptane	217
Alkyne	$\text{C}_5\text{H}_{11}\text{C}\equiv\text{CCH}_3$	n-Heptane	178, 196, 225
Carbonyl	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3\text{CCH}_3 \end{array}$	n-Heptane	186, 280
	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3\text{COH} \end{array}$	Ethanol	204
Amido	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3\text{CNH}_2 \end{array}$	Water	214
Azo	$\text{CH}_3\text{N}=\text{NCH}_3$	Ethanol	339
Nitro	CH_3NO_2	Isooctane	280
Nitroso	$\text{C}_4\text{H}_9\text{NO}$	Ethyl ether	300, 665
Nitrate	$\text{C}_2\text{H}_5\text{ONO}_2$	Dioxane	270

Jarzabek et al.²⁰ synthesized Schiff-base model compounds and characterized the different central groups and side-group of them to present the absorbance spectra using UV/vis spectroscopy. From the UV/vis spectrum, these compounds in different solvents show two distinct absorption bands, however with different intensity. The position of methyl group of these compounds affected their spectroscopic intensities and conjugation of π -electrons fundamentally.

2.6 General Experimental Section

2.6.1 Chemicals

Tocol,^{21, 22} the model tocopherols²³⁻²⁶ and Bu_4NPF_6 ²⁷ were prepared by standard methods. 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, recrystallizing 3 times from hot ethanol followed by drying under vacuum at 160 °C for 24 hours and storing under vacuum. Lecithin (refined), 1-octadecanethiol (96%) and TTF (tetrathiafulvalene) (97%) were obtained from Alfa Aesar, TCNQ (tetracyanoquinodimethane) ($\geq 98\%$) was obtained from Sigma-Aldrich, potassium hexacyanoferrate (II) trihydrate ($\geq 99\%$) and potassium hexacyanoferrate (III) ($\geq 99\%$) were purchased from Merck, and potassium chloride (AR grade) was obtained from Fisher Scientific. $(\pm)\text{-}\alpha\text{-TOH}$ (97%) was obtained from Aldrich and stored in the dark under nitrogen. The α -tocopherol quinone model compound, 2-(3-hydroxy-3-methylbutyl)-3,5,6-trimethylcyclohexa-2,5-diene-1,4-dione, was prepared by a literature procedure.^{28, 29} Nafion (5 wt. % in a mixture of lower aliphatic alcohols and containing 15 – 20% water) was obtained from Sigma-Aldrich. HPLC grade CH_3CN , CH_2Cl_2 , dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were used as received.

Chloroform (CHCl_3 , AR grade) and ethanol (AR grade) were from Fisher Scientific, and sulfuric acid (AR grade) was obtained from QReC. Water, with a resistivity $\geq 18 \text{ M}\Omega \text{ cm}$ from an ELGA Purelab Option-Q was used for experiments at different pH-values. Citric acid–phosphate buffer solutions (pH 3, 5 and 7) were prepared from disodium hydrogen phosphate (Merck) and citric acid (Amresco). Britton–Robinson buffer solutions (pH 3, 5, 7, 9, 11, and 13) were prepared using 0.04 M acetic, phosphoric, and boric acids (Merck), and adjusted to the required pH using NaOH (Merck).

2.6.2 Instrumental procedures

Cyclic voltammetric (CV) experiments were conducted with a computer controlled Eco Chemie Autolab PGSTAT 100 with an ADC fast scan generator. In organic solutions, working electrodes were 1 mm, 0.1 mm, 50 μm and 20 μm diameter planar Pt disks, used in conjunction with a Pt auxiliary electrode and an Ag wire reference electrode connected to the test solution *via* a salt bridge containing 0.5 M Bu_4NPF_6 in CH_3CN or CHCl_3 . Accurate potentials were obtained using ferrocene as an internal standard. Variable temperature (243 – 313 K) experiments were controlled with a Thermo Electron Neslab RTE 740 circulating bath. In aqueous solutions, working electrodes were Metrohm 3 mm diameter planar Au or GC disks, used in conjunction with a Metrohm Pt wire auxiliary electrode and a Metrohm Ag/AgCl reference electrode containing 3 M KCl. Rotating disk electrode experiments were conducted with a Metrohm Autolab RDE with a 3 mm diameter planar Au or GC electrodes.

Reflectance FTIR experiments were conducted with a Thermo Electron Nicolet 6700 spectrometer mainframe with a Continuum infrared microscope.

UV-vis spectra were obtained with a PerkinElmer Lambda 750 spectrophotometer.

2.6.3 Theoretical calculations

Digital simulations of the CV data were performed using the DigiElch software package.³⁰⁻³⁵ Molecular orbital calculations were performed using a development version of the Q-Chem 2.1 software package³⁶ and the Spartan '04 software package.³⁷ Electrostatic potential maps and potential-derived atomic charges were computed by Spartan at the B3LYP/6-31G* level using structures optimized at the same level.

References:

1. Bond, A. M., *Broadening Electrochemical Horizons*. Oxford University Press Inc.: New York, 2002.
2. Bond, A. M., *Modern polarographic methods in analytical chemistry*. Marcel Dekker: New York, 1980.
3. Bard, A. J.; Faulkner, L. R., *Electrochemical methods: fundamentals and applications*. John Wiley: New York, 1st edition 1980, 2nd edition 2001.
4. Bond, A. M.; Henderson, T. L. E.; Mann, D. R.; Mann, T. F.; Thormann, W.; Zoski, C. G., *J. Am. Chem. Soc.* **1988**, 60, 1882-1886.
5. Hamann, C. H.; Hamnett, A.; Vielstich, W., *Electrochemistry*. Wiley-Vch: Weinheim, 1998.
6. Stulik, K.; Amatore, C.; Holub, K.; Marecek, V.; Kutner, W., *Pure Appl. Chem.* **2000**, 72, 1483-1492.
7. Wight, R. M.; Wipf, D. O., *Acc. Chem. Res.* **1990**, 23, 64-70.
8. Pletcher, D., *A first courses in electrode processes*. Electrochemical consultancy: Hants, England, 1991
9. Nikolic, J.; Exposito, E.; Iniesta, J.; Gonzalez-Garcia, J.; Montiel, V., *J. Chem. Edu.* **2000**, 77, 1191-1194.
10. Cooper, J. A.; Compton, R. G., *Electroanalysis* **1998**, 10, 141-155.
11. Finkelstein, D. A.; Mota, N. D.; Cohen, J. L.; Abruna, H. D., *J. Phys. Chem. C* **2009**, 113, 19700-19712.
12. Bell, R. J., *Introductory Fourier Transform Spectroscopy*. Academic press: New York, 1972.

13. Griffiths, P. R., *Chemical Infrared Fourier Transform Spectroscopy*. Wiley-Interscience: New York, 1975.
14. Koenig, J. L., *Acc. Chem. Res.* **1981**, 14, 171-178.
15. www.thermo.com.
16. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R., *Foundamentals of Analytical Chemistry*. Thomson Brooks/Cole: USA, 8th edition, 2004.
17. Hishikawa, Y.; Kataoka, Y.; Kondo, T., *Abstracts of papers of the American Chemical Society* **1996**, 212, 47- cell.
18. Zecchina, A.; Bertarione, S.; Damin, A.; Scarano, D.; Lamberti, C.; Prestipino, C.; Spoto, G.; Bordiga, S., *Phys. Chem. Chem. Phys.* **2003**, 5, 4414-4417.
19. Denney, R. C.; Sinclair, R., *Visible and Ultraviolet Spectroscopy*. John Wiley & Sons: London, 1987.
20. Jarzabek, B.; Kaczmarczyk, B.; Sek, D., *Spectrochimica Acta Part A* **2009**, 74, 949-957.
21. Wang, Q.; She, X.; Ren, X.; Ma, J.; Pan, X., *Tetrahedron: Asymmetry* **2004**, 15, 29-34.
22. Ismail, F. M. D.; Hilton, M. J.; Stefinovic, M., *Tetrahedron Lett.* **1992**, 33, 3795-3796, and references cited within.
23. Smith, L. I.; Ungnade, H. E.; Hoehn, H. H.; Wawzonek, S., *J. Org. Chem.* **1939**, 4, 311-317.
24. Smith, L. I.; Tess, R. W. H., *J. Am. Chem. Soc.* **1944**, 66, 1523-1525.
25. Lien, E. J.; Ren, S. J.; Bui, H. Y. H.; Wang, R. B., *Free Radical Biol. Med.* **1999**, 26, 285-294.

26. Wright, J. S.; Johnson, E. R.; DiLabio, G. A., *J. Am. Chem. Soc.* **2001**, 123, 1173-1183.
27. Kissinger, P. T.; Heineman, W. R., Marcel Dekker: New York, 1984.
28. Patel, A.; Netscher, T.; Gille, L.; Mersiter, K.; Rosenau, T., *Tetrahedron* **2007**, 63, 5312-5318.
29. Peng, H. M.; Webster, R. D., *J. Org. Chem.* **2008**, 73, 2169-2175.
30. Rudolph, M., *J. Electroanal. Chem.* **2003**, 543, 23-29.
31. Rudolph, M., *J. Electroanal. Chem.* **2004**, 571, 289-307.
32. Rudolph, M., *J. Electroanal. Chem.* **2003**, 558, 171-176.
33. Rudolph, M., *J. Comput. Chem.* **2005**, 206, 619-632.
34. Rudolph, M., *J. Comput. Chem.* **2005**, 26, 633-641.
35. Rudolph, M., *J. Comput. Chem.* **2005**, 26, 1193-1204.
36. Shao, Y.; Molnar, L. F.; Jung, Y.; Kussmann, J.; Ochsenfeld, C.; Brown, S. T.; Gilbert, A. T. B.; Slipchenko, L. V.; Levchenko, S. V.; O'Neill, D. P.; Jr., R. A. D.; Lochan, R. C.; Wang, T.; Beran, G. J. O.; Besley, N. A.; Herbert, J. M.; Lin, C. Y.; Voorhis, T. V.; Chien, S. H.; Sodt, A.; Steele, R. P.; Rassolov, V. A.; Maslen, P. E.; Korambath, P. P.; Adamson, R. D.; Austin, B.; Baker, J.; Byrd, E. F. C.; Dachsel, H.; Doerksen, R. J.; Dreuw, A.; Dunietz, B. D.; Dutoi, A. D.; Furlani, T. R.; Gwaltney, S. R.; Heyden, A.; Hirata, S.; Hsu, C.-P.; Kedziora, G.; Khalliulin, R. Z.; Klunzinger, P.; Lee, A. M.; Lee, M. S.; Liang, W.; Lotan, I.; Nair, N.; Peters, B.; Proynov, E. I.; Pieniazek, P. A.; Rhee, Y. M.; Ritchie, J.; Rosta, E.; Sherrill, C. D.; Simmonett, A. C.; Subotnik, J. E.; Woodcock, H. L. I.; Zhang, W.; A. T. Bell; Chakraborty, A. K.; Chipman, D. M.; Keil, F.

J.; Warshel, A.; Hehre, W. J.; Schaefer, H. F. I.; Kong, J.; Krylov, A. I.; Gill, P. M. W.; Head-Gordon, M., *Phys. Chem. Chem. Phys.* **2006**, 8, 3172-3191.

37. *Spartan '04 for Macintosh*. Wavefunction Inc. Irvine CA.

Chapter 3

Variable Scan Rate Cyclic Voltammetry and Theoretical Studies on Tocopherol (Vitamin E) Model Compounds

3.1 Introduction

In this Chapter we discuss general fast scan cyclic voltammetry experiments that were performed on vitamin E in organic solvents. Vitamin E comprises a series of phenolic compounds (tocols, tocopherols and tocotrienols) that are produced by plants, some of which are essential to human health. In this thesis, vitamin E refers to the collection of tocol (H_3 -TOH) and tocopherols (α -, β -, γ -, and δ -TOH) that differ in their degree of methyl substitution around the phenolic ring (Scheme 3.1). There are currently two conflicting schools of thought regarding the biological function of vitamin E. One school takes the traditional and most widely accepted view that vitamin E's sole function is as an antioxidant, which is essentially a sacrificial compound that preferentially reacts with harmful free radicals thereby preventing lipid peroxidation (*via* a well-established radical termination mechanism).¹⁻⁴ The second theory is that vitamin E (primarily the α -tocopherol form) is involved in specific cellular signaling mechanisms, instead of, or in addition to, its antioxidant functions, although the mechanism at the molecular level is not currently understood.⁵⁻¹⁰ It is likely that the latter scenario (should it occur) also involves oxidised forms of vitamin E, therefore, electrochemical experiments can provide useful mechanistic information regarding potential *in vivo* reactions.

Scheme 3.1 Structures of the tocol (H₃-TOH) and tocopherol (α-, β-, γ-, and δ-TOH) forms of vitamin E

^aTocol has no known biological activity. (Tocotrienol forms of vitamin E are similar but have an unsaturated phytyl tail in position 1')

The electrochemical behavior of the tocopherols in acetonitrile and dichloromethane solutions has been studied extensively¹¹⁻²⁰ and has recently been reviewed.¹⁸ The overall electrochemical mechanism can be considered to be similar to that proposed for other phenols²¹⁻²⁶ with some important exceptions (Scheme 3.2). In the absence of added acid or base, initial one-electron oxidation of the tocopherols (TOH) produces the cation radicals (TOH⁺), which lose a proton to form the phenoxyl radicals (TO[•]). Evidence for the initial charge transfer and chemical step occurring consecutively (separately) rather than concertedly (simultaneously),²⁷⁻²⁹ came from spectroscopic measurements in dry acidic conditions that allowed detection of the cation radical.^{11, 12, 14-20} In the absence of acid, the phenoxyl radicals (having less positive oxidation potentials than the starting phenols) undergo immediate further one-electron oxidation to form the phenoxonium cations (TO⁺) in an ECE mechanism.^{11, 12, 14-20} The principal differences observed in the electrochemical

behavior of the tocopherols, when compared to most other phenols, are that: (i) the oxidation reaction is chemically reversible on the CV timescale (and for the α and β forms is reversible on electrolysis time scales²⁰), and (ii) the cation radicals and phenoxonium cations are stable enough to be detected by cyclic voltammetry and in some cases by spectroscopy. The phenoxonium cation of α -tocopherol (α -TO⁺) is particularly stable and can even be isolated as a pure solid compound when crystallized with a non-nucleophilic counteranion.¹²

The mechanism in Scheme 3.2 has largely been determined by slow scan rate CV and controlled potential electrolysis experiments,^{11, 12, 14-20} combined with detailed spectroscopic analysis (EPR, FTIR, NMR and UV-vis spectroscopies and X-ray crystallography);^{11, 12, 15, 17-20} although there currently exists little kinetic data for the homogeneous chemical steps. This Chapter uses variable scan rate cyclic voltammetry between 0.1 – 500 V s⁻¹ to study kinetic aspects of the reactions that occur in Scheme 3.2, as well as the additional steps associated with secondary oxidation processes that occur for the less methylated tocol and tocopherols (H₃-, γ -, δ -TOH). In all cases the model compounds were used where the phytol tails were replaced with methyl groups (position 1' in Scheme 3.1); since these compounds can be synthesized in a higher degree of purity (than the oil-like natural compounds) and their electrochemical behavior has been shown to be identical to the natural compounds (Scheme 3.3).^{11, 15} The electrochemical experiments were complemented with results from high level theoretical calculations on the phenoxonium cations, in order to determine whether there was a relationship between the observed lifetimes in solution, and the structure and positive charge distribution within the phenoxonium cations.

^aOne resonance structure is displayed for each compound. The listed potentials (vs. ferrocene^{0/+}) were obtained by voltammetry and are the approximate values necessary to bring about oxidation of the phenolic compounds but do not necessarily correspond to the formal potential. The counter ions for the charged species are the supporting electrolyte cation [Bu_4N^+] and anion [PF_6^-], and the " H^+ " ions exist coordinated to the organic solvent. $R_1, R_2, R_3 = \text{H or CH}_3$. $R_4 = \text{CH}_3 \text{ or } (\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3))_3\text{CH}_3$.

Scheme 3.2 Electrochemically induced transformations of vitamin E and model compounds in dry CH_3CN or CH_2Cl_2 ^{11, 12, 15, 17-20 a}

Scheme 3.3 Structures of the model tocopherol and tocol compounds used in this Chapter where the phytyl tail is replaced with a methyl group

3.2 Results and Discussion

3.2.1 $(\text{CH}_3)\alpha\text{-TOH}$

Cyclic voltammograms (CVs) of $(\text{CH}_3)\alpha\text{-TOH}$ in CH_3CN over a range of scan rates are shown in Figure 3.1 (solid lines), along with the corresponding digital simulations (dashed lines). The CVs were modeled based on the pathway 2 mechanism in Scheme 3.2, where the forward positive potential scan involved the chemically reversible transformation of the phenol into the phenoxonium cation $[(\text{CH}_3)\alpha\text{-TO}^+]$ via two one-electron oxidation steps and an accompanying proton loss (an ECE mechanism). When the scan direction was reversed, a reductive peak was detected that was associated with the transformation of the phenoxonium cation back to the starting material. The data in Figure 3.1 illustrate that the ratio of the oxidative (i_p^{ox}) to reductive (i_p^{red}) peak currents ($i_p^{\text{ox}}/i_p^{\text{red}}$) remained approximately constant as the scan rate was increased, while the

anodic (E_p^{ox}) to cathodic (E_p^{red}) peak-to-peak separation (ΔE_{pp}) increased with increasing scan rate.

Figure 3.1 CVs of 2 mM $(\text{CH}_3)\alpha\text{-TOH}$ in CH_3CN with 0.5 M Bu_4NPF_6 at different scan rates (ν) at a 1 mm diameter Pt electrode. Current data were scaled by multiplying by $\nu^{-0.5}$. (—) Experimental data. (·····) Digital simulations (see parameters in Table 3.1)

Because the reduction potential of the cation radical ($\text{TOH}^{+\bullet}$) is greater than the oxidation potential of the phenoxyl radical ($\text{TO}^\bullet$) by approximately +0.4 V, the two compounds should undergo a homogeneous charge transfer reaction to form the

phenoxonium cation (TO^+) and regenerate the starting material (TOH) (Scheme 3.4). It is very difficult to experimentally determine whether the reaction in Scheme 3.4 (termed a disproportionation step³⁰⁻³⁷) occurs instead of the second heterogeneous charge transfer step, or whether the ECE mechanism, as illustrated as pathway 2 in Scheme 3.2, solely takes place. It is possible that both the mechanisms in Scheme 3.2 and 3.4 occur simultaneously, depending on whether the $\text{TO}^\bullet$ radical encounters the electrode surface (heterogeneous charge transfer) or encounters a cation radical (homogeneous charge transfer) and depending on the relative rates of the heterogeneous and homogeneous charge transfer steps. The electrochemical data were modeled only via the ECE mechanism. One observation that favors the simpler ECE mechanism is that the reaction is completely chemically reversible. In order for the disproportionation mechanism to occur, the equilibrium and rate constants for the C step and disproportionation step would both coincidentally need be "tuned" to allow the reactions to favorably progress in either direction.

Scheme 3.4 Homogeneous electron transfer reaction (disproportionation step)

It was possible to obtain a simulated voltammogram that very closely matched the experimental voltammogram for any individual scan rate, when modeled according to the pathway 2 mechanism in Scheme 3.2. However, no series of simulated voltammograms *exactly* matched the experimental data when a range of scan rates were used with the same

simulation parameters. Therefore, the simulation results presented in Figure 3.1 (and other Figures) represent the best average values, taking into account that data were collected over a large range of scan rates and at different sized electrodes.

Figure 3.2 shows the CVs obtained at electrodes of several sizes, demonstrating that at a scan rate of 100 V s^{-1} , the ΔE_{pp} -values remain approximately constant at the different electrodes. Considering that the effects of uncompensated solution resistance are the highest at the largest surface area electrode, the close similarity in the peak separations in the voltammograms in Figure 3.2 indicates that the increasing ΔE_{pp} -values shown in Figure 3.1 at faster scan rates are mainly the result of relatively slow heterogeneous electron transfer rates (k_s), rather than the effects of uncompensated solution resistance. By comparing the ΔE_{pp} -values obtained for ferrocene under identical conditions (where it was assumed that the k_s -value was $\geq 1 \text{ cm s}^{-1}$),³⁸ estimates on the solution resistance at varying temperatures were made and incorporated into the simulations.

Figure 3.3 shows CVs obtained of $(\text{CH}_3)\alpha\text{-TOH}$ at various temperatures, illustrating that the $i_p^{\text{ox}}/i_p^{\text{red}}$ -ratios and ΔE_{pp} -values remain close to constant as the temperature is changed. It was found that varying the scan rate between $0.1 - 500 \text{ V s}^{-1}$ while varying the temperature between $253 - 313 \text{ K}$ did not significantly alter the position (V) or ratio ($i_p^{\text{ox}}/i_p^{\text{red}}$) of the voltammetric waves beyond differences that could be assigned due to solution resistance or slow rates of heterogeneous electron transfer. Therefore, it can be concluded that the homogeneous proton transfer reaction in Scheme 3.2, that occurs between $(\text{CH}_3)\alpha\text{-TOH}^{+\bullet}$ and $(\text{CH}_3)\alpha\text{-TO}^\bullet$, occurs sufficiently quickly at all temperatures so as not to be outrun at scan rates $\leq 500 \text{ V s}^{-1}$ in CH_3CN solutions. Due to the number of steps involved in the oxidation process and because the homogeneous step occurred

Figure 3.2 CVs of 2 mM $(CH_3)\alpha$ -TOH in CH_3CN with 0.5 M Bu_4NPF_6 at 293 K at different sized Pt working electrodes. The scan rate = 100 V s^{-1} . (—) Experimental data. (.....) Digital simulations (see parameters in Table 3.1)

relatively quickly, it was possible to obtain simulations that matched the experimental data for a number of values of the forward ($k_{f(1)}$) and reverse ($k_{b(1)}$) rate constants (Scheme 3.5), with the range of values given in Table 3.1. The simulations indicate that the forward and reverse reactions are both fast, with the protonation rate approaching that of a diffusion controlled process.

Fig. 3.3 CVs of 2 mM (CH₃) α -TOH in CH₃CN with 0.5 M Bu₄NPF₆ at a scan rate of 0.5 V s⁻¹ at variable temperatures at a 1 mm diameter Pt electrode. (—) Experimental data. (·····) Digital simulations (see parameters in Table 3.1)

Scheme 3.5 Electrochemical oxidation mechanism of tocol and tocopherols studied by cyclic voltammetry over a range of temperatures and scan rates^a

^aElectrochemical, equilibrium and kinetic values associated with the heterogeneous and homogeneous steps are given in Tables 3.1 and 3.2. R₁, R₂, R₃ = H or CH₃.

Table 3.1 Equilibrium, rate constants and electrochemical parameters obtained by digital simulation of CV^a data for the ECE reaction given in Scheme 3.5 (pathway 2 in Scheme 3.2)

Compound	Solvent	$E_{f(1)}^0{}^b / \text{V}$	$E_{f(2)}^0{}^b / \text{V}$	$K_{eq(1)}{}^c / \text{mol L}^{-1}$	$k_{f(1)}{}^c / \text{s}^{-1}$	$k_{b(1)}{}^c / \text{L mol}^{-1} \text{s}^{-1}$
(CH ₃) α -TOH	CH ₃ CN	+0.50 $\pm$ 0.05	+0.15 $\pm$ 0.05	$1.2 (\pm 1.0) \times 10^{-4}$	$3.0 (\pm 2) \times 10^4$	$2.5 (\pm 2) \times 10^8$
(CH ₃) α -TOH	CH ₂ Cl ₂	+0.50 $\pm$ 0.05	+0.15 $\pm$ 0.05	$1.2 (\pm 1.0) \times 10^{-5}$	$3.0 (\pm 2) \times 10^5$	$2.5 (\pm 2) \times 10^{10}$
(CH ₃) γ -TOH	CH ₃ CN	+0.55 $\pm$ 0.05	+0.25 $\pm$ 0.05	$2.0 (\pm 1.5) \times 10^{-4}$	$3.0 (\pm 2) \times 10^3$	$1.5 (\pm 1) \times 10^7$
(CH ₃) δ -TOH	CH ₃ CN	+0.65 $\pm$ 0.05	+0.30 $\pm$ 0.05	$2.0 (\pm 1.5) \times 10^{-4}$	$5.0 (\pm 4) \times 10^4$	$2.5 (\pm 2) \times 10^8$
(CH ₃)H ₃ -TOH	CH ₃ CN	+0.75 $\pm$ 0.05	+0.40 $\pm$ 0.05	$1.7 (\pm 1.5) \times 10^0$	$2.5 (\pm 2) \times 10^5$	$1.5 (\pm 1) \times 10^6$

^aCV data recorded with 0.5 M *n*-Bu₄NPF₆ as the supporting electrolyte with 20 μm , 50 μm , 0.1 mm and 1 mm diameter Pt electrodes, at scan rates between 0.1 – 500 V s⁻¹ and at temperatures from 313 – 253 K. ^bFormal potential vs. Fc/Fc⁺. Heterogeneous rate constants were estimated to be between 0.1 – 0.3 cm s⁻¹. Diffusion coefficient values (2.5×10^{-5} cm² s⁻¹ at 313 K to 0.3×10^{-5} cm² s⁻¹ at 253 K) for the starting materials were estimated by simulation techniques (the same values were used for all the oxidised forms of the same compound). ^cHomogeneous equilibrium and rate constants. The range in these values reflects the error associated with the simulations due to the high number of variables and because the forward ($k_{f(1)}$) and reverse ($k_{b(1)}$) steps are very fast (see text).

The voltammetric results obtained in CH_2Cl_2 solutions over a variety of temperatures and scan rates were similar to the data obtained in CH_3CN , although were affected by the higher solution resistance associated with the low dielectric constant solvent. At slow scan rates $\leq 0.2 \text{ V s}^{-1}$, the CVs showed an additional reductive peak when the scan direction was reversed (Figure 3.4), but at faster scan rates the voltammograms were very similar in appearance to those obtained in CH_3CN solutions.

Figure 3.4 CVs of 2 mM $(\text{CH}_3)\alpha\text{-TOH}$ in different solvents with 0.5 M Bu_4NPF_6 at a scan rate of 0.1 V s^{-1} at a 1 mm diameter Pt electrode

The voltammograms obtained for (CH₃) α -TOH in dimethylformamide (DMF) and dimethylsulfoxide (DMSO) solutions were significantly different from those obtained in CH₃CN or CH₂Cl₂ (Figure 3.4). CVs obtained of (CH₃) α -TOH in DMF and DMSO solutions at scan rates up to 500 V s⁻¹ showed only a forward oxidative process with no evidence of a cathodic peak associated with reduction of the phenoxonium cation back to the phenol (as occurs in CH₃CN and CH₂Cl₂). Therefore, the CV results provide no evidence for the existence of the persistent phenoxonium cations in DMF and DMSO, which is an unexpected result since the electrochemical behavior of compounds in the aprotic solvents CH₃CN and DMF are usually alike.³⁹ A further observation was that the peak potentials for the oxidation process in DMF and DMSO were shifted to less positive potentials compared to in CH₃CN and CH₂Cl₂. The identity of the chemical process associated with the extra reduction peak is currently unknown. However, it is known that it is closely linked to the water content of the solvent. It is always present in solvents that have very low water concentration (such as CH₂Cl₂).

The i_p^{ox} -values for the oxidation process were much lower in DMF and DMSO compared to those recorded in CH₃CN and CH₂Cl₂ (Figure 3.4). The lower peak currents can partly be explained by decreasing diffusion coefficient values in the more viscous solvents DMF and DMSO. Coulometry data collected during exhaustive controlled-potential oxidative electrolysis experiments on (CH₃) α -TOH allowed the calculation of 2.2 electrons per molecule in DMF and over 4 electrons per molecule in DMSO solutions, indicating a complicated long-term oxidation mechanism. The small voltammetric peak detected at -1.0 V vs. Fc/Fc⁺ when the scan direction was reversed for (CH₃) α -TOH in DMSO or DMF (Figure 3.4), became larger during the electrolysis experiments indicating

that it was due to a secondary oxidation product (but not the phenoxonium cation). By comparison with an authentic sample it was determined that the species responsible for the peak at -1.0 V was not the α -tocopherol quinone, which is known to be a long term oxidation product.⁴⁰⁻⁴³ Therefore, it appears that the oxidation process in DMF and DMSO occurs through a more complicated mechanism than that shown in Scheme 3.2.

3.2.2 $(\text{CH}_3)\gamma$ -TOH, $(\text{CH}_3)\delta$ -TOH and $(\text{CH}_3)\text{H}_3$ -TOH

The cyclic voltammograms obtained for the tocopherols and tocol in CH_3CN , with fewer methyl groups in the aromatic ring, were more complex than the fully methylated form. Figure 3.5 shows cyclic voltammograms obtained for $(\text{CH}_3)\gamma$ -TOH over a range of scan rates. (Experimental data for $(\text{CH}_3)\delta$ -TOH and $(\text{CH}_3)\text{H}_3$ -TOH showed similar features.) At a scan rate of 0.1 V s^{-1} , a reductive peak was detected when the scan direction was reversed at $-0.3 \text{ V vs. Fc/Fc}^+$. When the scan rate was increased to 10 V s^{-1} , the reductive peak at $-0.3 \text{ V vs. Fc/Fc}^+$ disappeared while concomitantly a reductive peak at $+0.2 \text{ V vs. Fc/Fc}^+$ increased in magnitude. The reductive peak at $+0.2 \text{ V vs. Fc/Fc}^+$ is associated with reduction of the phenoxonium cation back to the starting material. Thus, at a scan rate of 10 V s^{-1} , the cyclic voltammogram of $(\text{CH}_3)\gamma$ -TOH appeared very similar to the CV of $(\text{CH}_3)\alpha$ -TOH at a scan rate of 0.1 V s^{-1} (compare Fig 3.1 and 3.5). The voltammetric behavior of $(\text{CH}_3)\gamma$ -TOH can be rationalized by its phenoxonium cation formed by electrochemical oxidation being relatively short-lived, so at slower scan rates, the $i_p^{\text{ox}}/i_p^{\text{red}}$ -ratio for the phenol/phenoxonium cation transformation was $\gg 1$.

Figure 3.5 CVs of 2 mM $(\text{CH}_3)\gamma\text{-TOH}$ in CH_3CN with 0.5 M Bu_4NPF_6 at different scan rates (ν) at a 1 mm diameter Pt electrode. Current data were scaled by multiplying by $\nu^{-0.5}$. (—) Experimental data. (·····) Digital simulations (see parameters in Tables 3.1 and 3.2)

Table 3.2 Equilibrium, rate constants and electrochemical parameters obtained by digital simulation of CV^b data for the follow-up reactions given in Scheme 3.5^a

Compound	<i>T</i> / K	$E_{f(3)}^0$ ^b / V	$K_{eq(2)}$ ^c / L mol ⁻¹	$k_{f(2)}$ ^c / L mol ⁻¹ s ⁻¹	$k_{b(2)}$ ^c / s ⁻¹	$K_{eq(3)}$ ^c	$k_{f(3)}$ ^c / s ⁻¹	$k_{b(3)}$ ^c / s ⁻¹
(CH ₃)γ-TOH	313	-0.23	1 × 10 ³	3 × 10 ¹	3 × 10 ⁻²	5 × 10 ²	0.5	1 × 10 ⁻³
	293	-0.25	1 × 10 ³	3 × 10 ¹	3 × 10 ⁻²	5 × 10 ²	0.1	2 × 10 ⁻⁴
	273	-0.25	1 × 10 ³	8 × 10 ⁰	8 × 10 ⁻³	5 × 10 ²	0.1	2 × 10 ⁻⁴
	263	-0.27	1 × 10 ³	8 × 10 ⁰	8 × 10 ⁻³	5 × 10 ²	0.1	2 × 10 ⁻⁴
(CH ₃)δ-TOH	313	-0.20	2 × 10 ²	7 × 10 ¹	4 × 10 ⁻¹	5 × 10 ²	0.5	1 × 10 ⁻³
	293	-0.20	2 × 10 ²	5 × 10 ¹	3 × 10 ⁻¹	5 × 10 ²	0.1	2 × 10 ⁻⁴
	273	-0.25	2 × 10 ²	4 × 10 ¹	2 × 10 ⁻¹	5 × 10 ²	0.1	2 × 10 ⁻⁴
	263	-0.25	2 × 10 ²	3 × 10 ¹	2 × 10 ⁻¹	5 × 10 ²	0.1	2 × 10 ⁻⁴
(CH ₃)H ₃ -TOH	313	-0.12	1 × 10 ³	2 × 10 ²	2 × 10 ⁻¹	5 × 10 ²	1.0	2 × 10 ⁻³
	293	-0.12	1 × 10 ³	1 × 10 ²	1 × 10 ⁻¹	5 × 10 ²	0.1	2 × 10 ⁻⁴

^a Data are not available for (CH₃)α-TOH because the processes occur slower than the cyclic voltammetric timescale. ^bCV data recorded in CH₃CN (with 0.5 M *n*-Bu₄NPF₆), at 20 μm, 50 μm, 0.1 mm and 1 mm diameter Pt electrodes, at scan rates between 0.1 – 500 V s⁻¹ and at temperatures from 313 – 263 K. ^bFormal potential vs. Fc/Fc⁺ reported to the nearest 10 mV. Heterogeneous rate constants were estimated to be between 0.1 – 0.3 cm s⁻¹. Diffusion coefficient values (2.5 × 10⁻⁵ cm² s⁻¹ at 313 K to 0.5 × 10⁻⁵ cm² s⁻¹ at 263 K) for the starting materials were estimated by simulation techniques (the same values were used for all the oxidised forms of the same compound). ^cHomogeneous equilibrium and rate constants reported to one significant figure.

The additional reductive peak at $-0.3\text{ V vs. Fc/Fc}^+$ observed at slow scan rates during the oxidation of $(\text{CH}_3)\gamma\text{-TOH}$ is due to a reaction product of the phenoxonium cation, and has been assigned as a hemiketal [**TOQ(OH)**], which subsequently converts into the *para*-quinone (**TOQ**) (Scheme 3.5).^{14-16, 40-43} Water is usually present in mM concentrations even in "dry" organic solvents, hence the hemiketal forms by a hydrolysis reaction of the reactive phenoxonium cation with trace water. The hemiketal is itself not stable and further isomerizes to form the *para*-quinone (Scheme 3.5, **TOQ**). Evidence for the isomerization reaction (Scheme 3.5, $k_{f(3)}$) is detectable in the voltammograms at slow scan rates, because the peak at $-0.3\text{ V vs. Fc/Fc}^+$ (due to the hydrolysis product) initially increases in size as the scan rate is increased from 0.1 to 1 V s^{-1} , due to the isomerization reaction being outrun. At scan rates faster than $\sim 1\text{ V s}^{-1}$, the hydrolysis reaction (Scheme 3.5, $k_{f(2)}$) is also outrun, hence the peak at -0.3 V diminishes in size until it is undetectable at $\nu = 10\text{ V s}^{-1}$.

The rate constants for the secondary processes [$k_{f(2)}$, $k_{b(2)}$, $k_{f(3)}$ and $k_{b(3)}$] that occur for $(\text{CH}_3)\gamma\text{-TOH}$, $(\text{CH}_3)\delta\text{-TOH}$ and $(\text{CH}_3)\text{H}_3\text{-TOH}$ (Scheme 3.5) were much slower than the proton transfer step [$k_{f(1)}$ and $k_{b(1)}$], thus systematic differences could be observed in the voltammograms as the temperature was changed. Figure 3.6 shows CVs of $(\text{CH}_3)\gamma\text{-TOH}$ at several temperatures (the CVs of $(\text{CH}_3)\delta\text{-TOH}$ and $(\text{CH}_3)\text{H}_3\text{-TOH}$ were qualitatively similar). The most obvious change to the voltammograms was that the peak for the reduction of the phenoxonium cation became larger as the temperature was lowered, due to its increased lifetime (Figure 3.6). Concomitantly to the peak for the reduction of the phenoxonium cation becoming larger at low temperature, the peak for the reduction of the hemiketal at $-0.3\text{ V vs. Fc/Fc}^+$ [**TOQ(OH)**], became smaller.

Figure 3.6 CVs of 2 mM $(\text{CH}_3)_7\text{-TOH}$ in CH_3CN with 0.5 M Bu_4NPF_6 at a scan rate of 0.5 V s^{-1} at variable temperatures at a 1 mm diameter Pt electrode. (—) Experimental data. (.....) Digital simulations (see parameters in Tables 3.1 and 3.2)

The entire oxidation mechanism for the phenols is thus given in Scheme 3.5, with the corresponding kinetic data obtained by digital simulations summarized in Tables 3.1 and 3.2. For (CH₃)_α-TOH, only the first 3 steps occur (two one-electron transfers and one proton transfer) on the CV timescale, to reversibly form the phenoxonium cation (TO⁺). However, for the less methylated compounds, $k_{f(2)}$ and $k_{f(3)}$ are sufficiently fast to be detectable by CV experiments and so affect the appearance of the voltammograms as the scan rate and temperature are varied. The large number of steps in the overall mechanism meant that the rate and equilibrium constants for the secondary chemical steps could only be estimated to one significant figure (Table 3.2).

3.2.3 Discussion on electrochemical data

Some conclusions can be drawn from the electrochemical, equilibrium and kinetic values presented in Tables 3.1 and 3.2. The effect of the electron donating methyl groups can be seen in the first and second electron transfer steps (Table 3.1; $E^0_{(1)}$ and $E^0_{(2)}$) which shift to more positive potentials with decreasing number of methyl groups in the aromatic ring, so that the one-electron oxidation potentials increase in the order (CH₃)_α-TOH < (CH₃)_γ-TOH < (CH₃)_δ-TOH < (CH₃)_{H3}-TOH.

Another observation from the digital modeling studies is that the equilibrium constant for the proton transfer ($K_{eq(1)}$) is < 1 mol L⁻¹, which accounts for the ECE portion of the mechanism being chemically reversible for all of the compounds at all measured scan rates. Furthermore, the chemical reversibility of the protonation reaction is assisted by the second order rate constant ($k_{b(1)}$) being very fast (approaching diffusion controlled for (CH₃)_α-TOH). One explanation for the very fast protonation reaction would be the occurrence of a proton tunneling mechanism,⁴⁴ which has been shown to take place for vitamin E when the phenolic starting material (α-TOH) reacts with an oxidised site on a lipid cell wall (ROO[•]) to form the phenoxyl

radical (α -TO $\cdot$) and lipid hydroperoxide (ROOH).⁴⁵⁻⁴⁸ Other organic cation radicals have also been shown to undergo proton tunneling mechanisms in CH₃CN.⁴⁹ It is important that the protonation reaction is fast, because the TO $\cdot$ radicals are known to decay by a bimolecular self-reaction with a rate constant of around $1 \times 10^3 \text{ L mol}^{-1} \text{ s}^{-1}$ (for α -TO $\cdot$) in acetonitrile at 293 K,¹⁷ although the values vary in other solvents and with other tocopherols.^{3, 50-52} Thus, if the protonation reactions were slow, the TO $\cdot$ radicals would irreversibly react before the cations could be reduced back to starting material. The bimolecular decay reaction (Scheme 3.5, $k_{\text{bimolec}} = 1 \times 10^3 \text{ L mol}^{-1} \text{ s}^{-1}$) was included in the overall mechanism, although its inclusion did not affect the appearance of the simulated voltammograms.

The equilibrium constants for the follow-up chemical reactions ($K_{\text{eq}(2)}$ and $K_{\text{eq}(3)}$) for the less methylated tocopherols [(CH₃) γ -TOH and (CH₃) δ -TOH] and tocol ((CH₃)H₃-TOH) are > 1 (200-1000 L mol^{-1} and 500, respectively) indicating that the forward reactions (hydrolysis and isomerization, respectively) were favored (Table 3.2). The rate constants for the hydrolysis processes ($k_{\text{f}(2)}$) increased in the order (CH₃) α -TOH $<$ (CH₃) γ -TOH $\approx$ (CH₃) δ -TOH $<$ (CH₃)H₃-TOH, with decreasing methylation of the aromatic ring (Table 3.2). Therefore, it appears that one of the reasons for the long lifetime of the phenoxonium cation of α -TOH (and (CH₃) α -TOH) in CH₃CN, is that it is relatively stable towards the hydrolysis reaction ($k_{\text{f}(2)}$) that occurs for the less methylated tocols and tocopherols. The rate constant values for the hydrolysis and isomerization reactions [$k_{\text{f}(2)}$ and $k_{\text{f}(3)}$] decreased as the temperature was lowered.

A key factor in the appearance of the voltammetric responses is the moisture content of the solvent. Since the hydrolysis reaction is the major route of reaction of the phenoxonium cations in CH₃CN at mM concentrations, small amounts of water will affect the CVs and therefore, the kinetic values for the reactions in Scheme 3.5.

Nevertheless, since the CV experiments were all performed under identical conditions, the relative lifetimes of the phenoxonium cations given in Tables 3.1 and 3. 2 are valid. It is possible that the increased equilibrium solubility of water in DMSO and DMF could account for decreased lifetimes of the phenoxonium cations in those media. DMF and DMSO are both very difficult to dry, but it is uncertain if the extra wetness of the DMF and DMSO is sufficient to decrease the lifetime of α -TO⁺. Furthermore, it has been observed that adding small amounts (mM) of DMF or DMSO to solutions of α -TOH in CH₃CN, results in a loss of chemical reversibility of the oxidation process, suggesting that α -TO⁺ is reacting with the DMS/DMSO. Direct reactions of the cationic compounds with the solvents are also possible, especially in the case of DMSO. DMSO undergoes a self-ionization reaction and its conjugate base (CH₃SOCH₂⁻) is a good nucleophile which may attack electrophilic centers. The α -tocopherol quinone product (**TOQ**) was not voltammetrically detected after oxidation of (CH₃) α -TOH in DMSO or DMF, suggesting that the long time-scale reaction(s) in DMSO and DMF were more complicated than hydrolysis of the phenoxonium cation. At high concentrations or in very low dielectric constant solvents such as benzene or chlorobenzene, dimeric compounds are often identified as oxidation products of tocopherols.^{3, 43}

The addition of strong organic acids, such as CF₃SO₃H or CF₃COOH have a pronounced effect on the appearance of the voltammograms.^{14, 16, 17, 19, 20} As well as increasing the proton concentration, the acid also decreases the moisture content of the solvent and thereby changes the solvent composition (CF₃SO₃H reacts with H₂O to form the monohydrate, CF₃SO₃H•H₂O⁵³). It has been shown that reactive cation radicals (such as C₆₀⁺⁺)⁵⁴ are stabilized in organic solvents containing organic soluble

acids, by the acids reacting preferentially with trace nucleophiles (such as water), and the same situation can occur with TOH^{++} and TO^+ .²⁰

3.3 Theoretical calculations

One interesting question relates to why some phenoxonium cations have longer lifetimes in solution than others; in particular, large differences in lifetime exist between $\alpha\text{-TO}^+$ and the phenoxonium cations of the other tocots and tocopherols, despite relatively small differences in their structures. Therefore, the phenoxonium cations were investigated by molecular orbital calculations to determine their molecular structures and the positive charge distributions. The calculations were performed on the gas phase molecules, and do not take into account the more complex interactions that occur between the cations and the solvent molecules. Nevertheless, the theoretical results do indicate some interesting differences between the different tocopherol phenoxonium ions.

It was known from the crystal structure of $(\text{CH}_3)\alpha\text{-TO}^+$,²⁰ that the bond distance between the oxygen atom and the quaternary carbon atom ($\text{C}_{12}\text{-O}_{16}$ in Table 3.3) was relatively long for a carbon-oxygen bond (1.52 Å compared to 1.44 Å for a normal C-O bond). It was rationalized that the chromanol ring structure helped to maintain the $\text{C}_{12}\text{-O}_{16}$ bond intact despite it being long and therefore, weak. The results in Table 3.3 indicate that the length of the $\text{C}_{12}\text{-O}_{16}$ bond in the phenoxonium cations do increase as the degree of methyl substitution decreases. The calculations also indicate that as the $\text{C}_{12}\text{-O}_{16}$ bond length increases between the different phenoxonium cations, the $\text{C}_4\text{-O}_{16}$ bond length correspondingly decreases.

Table 3.3 Results from theoretical calculations at the B3LYP/6-31G* level for the phenoxonium cations of the tocopherols

Parameter	(CH ₃) α -TO ⁺	(CH ₃) β -TO ⁺	(CH ₃) γ -TO ⁺	(CH ₃) δ -TO ⁺	(CH ₃)H ₃ -TO ⁺
$r(\text{C}_{12}\text{-O}_{16}) / \text{\AA}^a$	1.520	1.524	1.529	1.536	1.539
$r(\text{C}_4\text{-O}_{16}) / \text{\AA}^a$	1.293	1.289	1.289	1.285	1.283
Charge on C ₁ ^b	+0.62	+0.56	+0.58	+0.67	+0.61
Charge on C ₂ ^b	+0.07	+0.14	−0.13	−0.11	−0.05
Charge on C ₃ ^b	−0.45	−0.23	+0.03	+0.03	−0.03
Charge on C ₄ ^b	+0.75	+0.34	+0.33	+0.32	+0.56
Charge on C ₅ ^b	−0.18	+0.26	+0.04	+0.27	−0.05
Charge on C ₆ ^b	−0.04	−0.18	+0.03	−0.17	+0.03
Charge on C ₇ ^b	+0.09	+0.05	-	-	-
Charge on C ₈ ^b	+0.08	−0.01	+0.04	−0.01	-
Charge on C ₉ ^b	+0.13	-	+0.08	-	-
Charge on C ₁₀ ^b	+0.32	+0.24	+0.14	+0.12	+0.12
Charge on C ₁₁ ^b	−0.22	−0.17	−0.09	−0.08	−0.10
Charge on C ₁₂ ^b	+0.93	+0.86	+0.78	+0.76	+0.82
Charge on C ₁₃ ^b	−0.11	−0.11	−0.10	−0.08	−0.10
Charge on C ₁₄ ^b	−0.14	−0.14	−0.14	−0.12	−0.11
Charge on O ₁₅ ^b	−0.38	−0.33	−0.34	−0.36	−0.35
Charge on O ₁₆ ^b	−0.46	−0.28	−0.24	−0.25	−0.35
Total Charge ^b	+1.01	+1.00	+1.01	+0.99	+1.00

^aBond length. ^bElectrostatic potential-derived charges. Atomic numbering system is given in Scheme 3.6.

Scheme 3.6 Atomic numbering system for the theoretical values given in Table 3.3^a

^aHydrogen bond are omitted for clarity.

Table 3.3 contains the calculated electrostatic potential-derived charges, where the charges on the hydrogen atoms are absorbed into their parent carbons, which show a number of interesting differences between $(\text{CH}_3)\alpha\text{-TO}^+$ and the other phenoxonium cations. If the total positive charge in the aromatic ring is summed, the charge increases in the order $(\text{CH}_3)\alpha\text{-TO}^+$ (+0.77) < $(\text{CH}_3)\beta\text{-TO}^+$ (+0.89) $\approx$ $(\text{CH}_3)\gamma\text{-TO}^+$ (0.88) < $(\text{CH}_3)\delta\text{-TO}^+$ (+1.01) < $(\text{CH}_3)\text{H3-TO}^+$ (+1.07). It could be concluded that the increasing positive charge in the aromatic ring favours an increased rate of reaction with nucleophiles, and therefore, results in a decrease in lifetime in solution. However, the calculations also indicate that most of the positive charge in the aromatic ring is located on C₁ and C₄, and for $(\text{CH}_3)\alpha\text{-TO}^+$, the positive charge on C₄ is extremely high compared to the other phenoxonium cations. Considering that nucleophilic attack on C₄ is a major reaction route for the phenoxonium cations, the high positive charge on C₄ for $(\text{CH}_3)\alpha\text{-TO}^+$ seems counterintuitive towards its long lifetime in solution. This argument does not take into account the steric hindrance that the C₈ methyl group would be expected to have in preventing nucleophilic attack at C₄ [which is not present in $(\text{CH}_3)\text{H3-TO}^+$].

A further interesting result from the electrostatic potential-derived charges is that for $(\text{CH}_3)\alpha\text{-TO}^+$, the ether oxygen (Scheme 3.6, O_{16}) carries a high negative charge compared to the other phenoxonium cations (-0.46 compared to between -0.24 to -0.35) (Table 3.3). The high negative charge on O_{16} could provide a mechanism for electrostatic repulsion for incoming nucleophiles, thereby decreasing the solution phase reaction rate. The calculations predict that the negative charge on O_{16} in $(\text{CH}_3)\alpha\text{-TO}^+$ is even higher than on the carbonyl oxygen (O_{15}), while for the other compounds the negative charge on $\text{O}_{16} < \text{O}_{15}$ (except for $(\text{CH}_3)\text{H}_3\text{-TO}^+$ where the charges on the oxygen atoms are equal). Therefore, for $(\text{CH}_3)\alpha\text{-TO}^+$, the anisotropic effect of the carbonyl group ($\text{C}_1\text{-O}_{15}$) hinders nucleophilic attack from the direction of the carbonyl group, the steric effect of the C_8 methyl group prevents nucleophilic attack across the open side double bond, and the electronegative oxygen (O_{16}) hinders nucleophilic addition from the direction of the chromanol ring.

3.4 Conclusions

The electrochemical oxidation of tocol and the tocopherols in CH_3CN and CH_2Cl_2 initially proceeds *via* an ECE mechanism to form phenoxonium cations. Digital simulation of variable scan rate cyclic voltammetric data allowed an estimation of the rate constants of the electrochemical and chemical steps. The rate constants for the protonation reaction (C-step) are fast, resulting in equilibrium constants that favor the oxidation processes being chemically reversible. The phenoxonium cations of the less methylated compounds (tocol and γ - and δ -tocopherol) are able to undergo a hydrolysis reaction on the voltammetric time-scale to form hemiketals which isomerize into *para*-quinones. The rate constants for the hydrolysis reaction ($k_{\text{H}(2)}$) increase with decreasing methylation of the aromatic ring [$(\text{CH}_3)\alpha\text{-TO}^+ < (\text{CH}_3)\gamma\text{-TO}^+ \approx (\text{CH}_3)\delta\text{-TO}^+ < (\text{CH}_3)\text{H}_3\text{-TO}^+$]. In contrast to CH_3CN and CH_2Cl_2 , the oxidation of

(CH₃) α -TOH in DMF and DMSO showed no evidence of chemical reversibility at scan rates up to 500 V s⁻¹, indicating that the lifetime of the phenoxonium cation was considerably less (than in CH₃CN and CH₂Cl₂).

The results from theoretical calculations indicated a number of systematic differences between the different tocopherols in terms of atomic bond lengths and charges. The reason for the increased lifetime in solution for (CH₃) α -TO⁺, compared to the other phenoxonium cations, is likely to be because of a complicated mixture of the charge distribution (resulting in electrostatic repulsions) and steric hindrance, effecting its interactions with nucleophiles.

References:

1. Traber, M. G.; Atkinson, J., *Free Radical Biol. Med.* **2007**, 43, 4-15.
2. Burton, G. W.; Ingold, K. U., *Acc. Chem. Res.* **1986**, 19, 194-201.
3. Bowry, V. W.; Ingold, K. U., *J.Org.Chem.* **1995**, 60, 5456-5467.
4. Bowry, V. W.; Ingold, K. U., *Acc. Chem. Res.* **1999**, 32, 27-34.
5. Azzi, A.; Stocker, A., *Prog. Lipid Res.* **2000**, 39, 231-255.
6. Ricciarelli, R.; Zingg, J.-M.; Azzi, A., *Biol. Chem* **2002**, 383, 457-465.
7. Azzi, A.; Ricciarelli, R.; Zingg, J.-M., *FEBS Lett.* **2002**, 519, 8-10.
8. Melton, L., *New Sci* **2006**, 191, 40-43.
9. Azzi, A., *Free Radical Biol. Med.* **2007**, 43, 16-21.
10. Azzi, A., *Biochem. Biophys. Res. Commun.* **2007**, 362, 230-232.
11. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D., *J.Org.Chem.* **2005**, 70, 10466-10473.
12. Lee, S. B.; Willis, A. C.; Webster, R. D., *J. Am. Chem. Soc.* **2006**, 128, 9332-9333.
13. Nanni, E. J.; Jr.; Stallings, M. D.; Sawyer, D. T., *J. Am. Chem. Soc.* **1980**, 102, 4481-4485.
14. Parker, V. D., *J. Am. Chem. Soc.* **1969**, 91, 5380-5381.
15. Peng, H. M.; Webster, R. D., *J. Org. Chem.* **2008**, 73, 2169-2175.
16. Svanholm, U.; Bechgaard, K.; Parker, V. D., *J. Am. Chem. Soc.* **1974**, 96, 2409-2413.
17. Webster, R. D., *Electrochem. Commun.* **1999**, 1, 581-584.
18. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
19. Williams, L. L.; Webster, R. D., *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.

20. Wilson, G. J.; Lin, C. Y.; Webster, R. D., *J. Phys. Chem. B* **2006**, 110, 11540-11548.
21. Eickhoff, H.; Jung, G.; Rieker, A., *Tetrahedron* **2001**, 57, 353-364.
22. Lund, H.; Hammerich, O., *Organic Electrochemistry 4th ed.* Marcel Dekker: New York, 2001.
23. Rieker, A.; Beisswenger, R.; Regier, K., *Tetrahedron* **1991**, 47, 645-654.
24. Speiser, B.; Rieker, A., *J. Chem. Res. Synop.* **1977**, 314, 315.
25. Speiser, B.; Rieker, A., *J. Electroanal. Chem.* **1979**, 102, 373-395.
26. Speiser, B.; Rieker, A., *J. Electroanal. Chem.* **1980**, 110, 231-246.
27. Costentin, C.; Robert, M.; Saveant, J.-M., *Chem. Phys.* **2006**, 324, 40-56.
28. Costentin, C.; Robert, M.; Saveant, J.-M., *J. Am. Chem. Soc.* **2006**, 128, 4552-4553.
29. 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.
30. Alden, J. A.; Compton, R. G., *J. Phys. Chem. B* **1997**, 101, 9606-9696.
31. Amatore, C.; Saveant, J. M., *J. Electroanal. Chem.* **1980**, 107, 353-364.
32. Compton, R. G.; Coles, B. A.; Pilkington, M. B. G.; Bethell, D., *J. Chem. Soc. Faraday Trans.* **1990**, 86, 663-670.
33. Compton, R. G.; Dryfe, R. A. W., *J. Electroanal. Chem.* **1994**, 375(1-2), 247-255.
34. Compton, R. G.; Pilkington, M. B. G.; Stearn, G. M., *J. Chem. Soc. Faraday Trans. I* **1988**, 84, 2155-2171.
35. Leslie, W. M.; Alden, J. A.; Compton, R. G.; Silk, T., *J. Phys. Chem* **1996**, 100, 14130-14136.

36. Leslie, W. M.; Compton, R. G.; Silk, T., *Electrochim. Acta* **1997**, 42, 3575-3584.
37. Neudeck, A.; Dittrich, J., *J. Electroanal. Chem.* **1989**, 264, 91-119.
38. Bond, A. M.; Henderson, T. L. E.; Mann, D. R.; Mann, T. F.; Thormann, W.; Zoski, C. G., *Anal. Chem.* **1988**, 60, 1878-1882.
39. Lund, H.; Hammerich, O., *Organic Electrochemistry*, 4th ed. Marcel Dekker: New York, 2001.
40. Durckheimer, W.; Cohen, L. A., *J. Am. Chem. Soc.* **1964**, 86, 4388-4393.
41. Marcus, M. F.; Hawley, M. D., *J. Org. Chem.* **1970**, 35, 2185-2190.
42. Patel, A.; Netscher, T.; Gille, L.; Mersiter, K.; Rosenau, T., *Tetrahedron* **2007**, 63, 5312-5318.
43. Rosenau, T.; Klosner, E.; Gille, L.; Mazzini, F.; Netscher, T., *J. Org. Chem.* **2007**, 72, 3268-3281.
44. Bell, R. P., *The Tunnel Effect in Chemistry*. Chapman and Hall: London, 1980.
45. Nagaoka, S.; Kuranaka, A.; Tsuboi, H.; Nagashima, U.; Mukai, K., *J. Phys. Chem* **1992**, 96, 2754-2761.
46. Nagaoka, S.; Sawada, K.; Fukumoto, Y.; Nagashima, U.; katsumata, S.; Mukai, K., *J. Phys. Chem* **1992**, 96, 6663-6668.
47. Nagaoka, S.; Mukai, K.; Itoh, T.; katsumata, S., *J. Phys. Chem* **1992**, 96, 8184-8187.
48. Nagaoka, S.; Inoue, M.; Nishioka, C.; Nishioku, Y.; Tsunoda, S.; Ohguchi, C.; Ohara, K.; Mukai, K.; Nagashima, U., *J. Phys. Chem. B* **2000**, 104, 856-862.
49. Lu, Y.; Zhao, Y.; Parker, V. D., *J. Am. Chem. Soc.* **2001**, 123, 5900-5907.
50. Doba, T.; Burton, G. W.; Ingold, K. U.; Matsuo, M., *J. Chem. Soc. Chem. Commun.* **1984**, 461, 462.

51. Rousseau-Richard, C.; Richard, C.; Martin, R., *FEBS Lett.* **1988**, 233, 307-310.
52. Watanabe, A.; Noguchi, N.; Fujisawa, A.; Kodama, T.; Tamura, K.; Cynshi, O.; Niki, E., *J. Am. Chem. Soc.* **2000**, 122, 5438-5442.
53. Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochman, M., *In Advanced Inorganic Chemistry*, 6th ed. Wiley-Interscience: New York, 1999.
54. Webster, R. D.; Heath, G. C., *Phys. Chem. Chem. Phys.* **2001**, 3, 2588-2594.

Chapter 4

Voltammetrically Controlled Electron Transfer Reactions from Alkanethiol Modified Gold Electrode Surfaces to Low Molecular Weight Molecules Deposited Within Lipid (Lecithin) Bilayers

4.1 Introduction

In this Chapter we discuss procedures that were developed for studying electron transfer reactions within biological membranes. The ultimate aim was to study biological molecules, but the initial experiments were performed on model compounds. While the electrochemical behavior of low molecular weight vitamins and coenzymes are often studied in organic solvents,¹⁻⁵ there still exists fundamental problems with studying electron transfer reactions under conditions similar to where they are known to occur naturally; that is, within a bilayer lipid membrane (BLM) environment.⁶⁻⁸ The methods that have been developed generally involve using supported bilayer lipid membranes (s-BLM); either attaching lipid layers to a solid substrate,⁹⁻¹³ or performing electrochemical measurements on supported lipid interfaces where the lipid bilayer separates two aqueous solutions.¹²⁻¹⁷ Due to difficulties in physically supporting the bilayers between two aqueous solutions, it is generally preferable to support the lipid layers on solid electrode surfaces, where the lipid bilayers are more robust.⁹⁻¹³

The majority of studies on electron transfer reactions that occur between electrode surfaces and species present within or attached to lipid bilayers have been performed on large macromolecules, such as proteins and DNA, which can span the entire bilayer structure.⁹⁻¹¹ One complication with small molecules that reside entirely within the lipid bilayer is that electron transfer reactions between the electrode and analyte must be balanced by the transfer of a suitable counter-ion across the bilayer-aqueous interface for the requirement of charge neutrality and for current to flow.

A procedure has been developed for preparing s-BLMs on electrode surfaces by immersing the solid electrode (usually Pt) in an aqueous solution containing liposomes and cutting the electrode to produce a fresh surface, which is thought to be less susceptible to oxidation and therefore, provides an improved metallic surface for coating.^{14, 15, 18, 19} Other procedures have been described where the solid electrode is simply polished in air, and a BLM is placed on top *via* deposition from a volatile solvent.^{9-11, 20-23} However, it is unlikely that the BLMs form a uniform defect free coating on metallic electrodes. The optimal surface for preparing s-BLMs is glass, where the lipid bilayers behave in a fluid manner and can be made to exist without defects in their structures.²⁴ Tethered bilayer lipid membranes (t-BLM) have been used in order to produce more uniform lipid coatings on solid electrodes, where one half of the bilayer is attached to the surface *via* a chemical linkage and the other half of the bilayer spontaneously forms from solution to attach itself to the tethered portion.²⁵⁻³⁷

There have been a number of studies where low molecular weight mediator compounds are incorporated into s-BLMs in order to promote electron transfer from the lipid bilayer to an aqueous solution phase species, with TCNQ identified as being particularly efficient as a mediator.³⁸⁻⁴⁶ Nevertheless, the electrochemical responses that are obtained are often difficult to interpret and are not usually as clearly defined as

those that are obtained for species dissolved in homogeneous aqueous or non-aqueous solvents.¹⁻⁵ In this Chapter it was found that in order to achieve reproducible electrochemical results with mediator compounds within s-BLMs on solid electrodes, it was necessary to prepare an alkanethiol self assembled monolayer (SAM) on the clean electrode surface first, and then deposit lipid multilayers.⁴⁶⁻⁴⁸ Thus, a solid Au electrode was used as the substrate with a monolayer of 1-octadecanethiol self assembled on the electrode surface. Multiple lipid bilayers of refined lecithin were then coated on the Au/alkanethiol electrode surface, with the organic mediators TCNQ or TTF incorporated into the lipid bilayers (Scheme 4.1). Lecithin (which mainly consists of phosphatidylcholine) was chosen for the lipid bilayers because it is a naturally occurring phospholipid present in plant and animal tissues. It will be demonstrated that TCNQ can act as an electron mediator for reactions that occur between the lipid bilayers and species in solution, and also exist as a stable radical anion inside the lipid (lecithin) multilayers.

Scheme 4.1 Structures of the molecules comprising the lipid multilayers

4.2 Results

4.2.1 Preparation of the Au/alkanethiol monolayer

The procedure was based on a literature report.⁴⁹ The gold electrodes were polished consecutively with P400 (35 μm) P1200 (15.3 μm) P2000 (10.3 μm) and P4000 (6.5 μm) grades of SiC paper followed by polishing with 3 μm and 1 μm grit alumina oxide powder on Buehler Ultra-Pad polishing cloths. The polished electrodes were sonicated in an ultrasonic bath in a beaker of water for 15 minutes followed by sonication in ethanol for a further 3 minutes. The electrodes were chemically cleaned at approximately 60 $^{\circ}\text{C}$ in 0.5 M sulfuric acid for 5 minutes, rinsed with water, and then 5 voltammetric scans were conducted between -0.3 to $+1.5$ V vs. Ag/AgCl in a freshly prepared deoxygenated solution of 0.5 M sulfuric acid at ~ 22 $^{\circ}\text{C}$. The electrode was rinsed with water then ethanol, dried under nitrogen and immersed in 5mM octadecanethiol in ethanol for 2 hours. The coated electrode was rinsed with ethanol and dried in a stream of nitrogen gas (Scheme 4.2a, 4.2b).

4.2.2 Preparation of the Au/alkanethiol/lecithin multilayer

A 15 mg mL^{-1} (1% w/w) solution of lecithin in chloroform was used to prepare two types of Au/alkanethiol/lecithin layers:^{12-14, 18, 19} (i) a multilayer containing only lecithin, (ii) a multilayer with a lecithin and TCNQ (or TTF) mixture. For multilayers without TCNQ, a 2- μL aliquot of the lecithin solution was dropped onto the SAM coated gold surface by micropipette and the chloroform was evaporated in air (Scheme 4.2c, 4.2d). The formation process of the lecithin multilayer containing TCNQ involved adding varying aliquots (50 to 500 μL) of a stock saturated solution of TCNQ in chloroform to a 500- μL volume of the lecithin solution, and then adding a 2- μL aliquot of the lecithin-TCNQ solution onto the alkylated gold electrode surface. UV-

visible experiments indicated that TCNQ is soluble up to approximately 5 mM in CHCl_3 at $22 (\pm 2)^\circ\text{C}$, enabling the amount of TCNQ within the lipid multilayers to be calculated. The same procedure was used to prepare lipids containing TTF, except the stock solution contained 10 mM TTF. Typically, the mass of lecithin on the electrode surface was $30\text{ }\mu\text{g}$, while the mass of TCNQ and TTF were $< 1\text{ }\mu\text{g}$.

Scheme 4.2 Electrode coating procedure (a) Cleaned and polished 3 mm diameter Au disk electrode. (b) Au electrode coated *via* self assembly with monolayer of octadecanethiol. (c) A $2\text{-}\mu\text{L}$ droplet of lecithin solution in CHCl_3 (containing TCNQ or TTF) placed on the electrode surface with a micropipettor. (d) After evaporation of the CHCl_3 to form the Au/alkanethiol/lecithin electrode

4.2.3 Voltammetry of TCNQ and TTF as solids and in solution phase

In order to understand the electrochemical responses obtained for TCNQ and TTF within lipid bilayers, it is useful to examine the electrochemical responses that are obtained when TCNQ and TTF are attached as solid layers to electrode surfaces and as solution phase species. Figure 4.1a shows CVs of a solid layer of TCNQ on Au that were obtained by allowing a $2\text{-}\mu\text{L}$ droplet of a saturated solution of TCNQ in CHCl_3 to evaporate, and placing the electrode in an aqueous solution containing 0.5 M KCl. In

this instance, the layer of TCNQ may consist of a film, as microcrystals, as amorphous particles or as a mixture of morphologies (in all cases the voltammetry appears the same).⁵⁰⁻⁵⁴ The CV is typical of solid state samples of TCNQ that show widely spaced reductive (E_p^{red}) and oxidative (E_p^{ox}) peaks, which correspond to the reversible formation of the radical anion and oxidation of the radical anion back to the starting material (Equation 4.1).⁵⁰⁻⁵⁹

In order to balance the increased negative charge during the reduction process, it is necessary for K^+ from the supporting electrolyte to be incorporated into the solid layer through a dissolution and nucleation process, which is thought to account for the large peak-to-peak separation (ΔE_{pp}) observed in the voltammograms.⁵⁰ Although $\text{TCNQ}^{\cdot-}$ is unreactive with water (and hence has a long lifetime) its salts are weakly soluble; therefore, some of the reduced material is lost from the electrode surface with repetitive scanning so that the anodic (i_p^{ox}) and cathodic (i_p^{red}) peak currents decrease with multiple scans (Figure 4.1a). A second one-electron reduction process is observed for solid samples of TCNQ if the potential is extended in the negative potential direction beyond $-0.75 \text{ V vs. Ag/AgCl}$ (data not shown).⁵⁰⁻⁵⁴

Figure 4.1b shows the CV of a saturated solution of TCNQ in CHCl_3 containing 0.5 M Bu_4NPF_6 as the supporting electrolyte. CHCl_3 was used as the solvent for the solution phase experiments because it is the solvent that is used to deposit the lecithin bilayers on the electrode surfaces in later studies. Furthermore, it has one of the lowest dielectric constants of solvents that still enable voltammetric experiments to be performed ($\epsilon = 5.5$); therefore, it is an interesting solvent system with which to compare the voltammetry that can be obtained in an even more challenging medium such as lecithin. The CV of a 5 mM solution of TCNQ shows two chemically

reversible reduction processes with large ΔE_{pp} -values of approximately 260 mV (compared to the minimum theoretical value of 57 mV at 25 °C⁶⁰). When the TCNQ concentration was decreased to 1 mM and 0.5 mM, the ΔE_{pp} -values decreased to 95 mV and 69 mV respectively. Therefore, the large ΔE_{pp} -values are due to the effects of uncompensated solution resistance brought about by the low dielectric constant of CHCl_3 , rather than a slow rate of heterogeneous electron transfer (it was found that decreasing the electrode size also lowered the ΔE_{pp} -values due to reducing the effects of uncompensated solution resistance). The voltammetric processes correspond to the one-electron reduction of TCNQ to $\text{TCNQ}^{\bullet-}$ at $E_{\text{obs}} = +0.25 \text{ V vs. Ag/AgCl}$ ($-0.19 \text{ V vs. ferrocene}^{0/+}$) and the further one-electron reduction of $\text{TCNQ}^{\bullet-}$ to TCNQ^{2-} at $-0.32 \text{ V vs. Ag/AgCl}$ ($-0.76 \text{ V vs. ferrocene}^{0/+}$).⁶¹ The E_{obs} -values were calculated from CV data under conditions where the ratio of $i_p^{\text{ox}}/i_p^{\text{red}}$ was equal to unity and using the relationship⁶⁰

$$E_{\text{obs}} = (E_p^{\text{red}} + E_p^{\text{ox}})/2 \quad (4.2)$$

Figures 4.1c and 4.1d show the corresponding solid state and solution phase cyclic voltammograms of TTF obtained under similar conditions as for TCNQ. The CV of solid samples of TTF is similar to that obtained for TCNQ (albeit the initial electron transfer reaction involves oxidation) with a wide separation between the forward and reverse peaks, due to the reversible incorporation of Cl^- into the $\text{TTF}/\text{TTF}^{+\bullet}$ layer (Equation 4.3).^{50, 62} The oxidation process observed during the first scan (Figure 4.1c, black line) occurs at a more positive potential than subsequent scans (red and blue lines). The peak currents observed for the oxidation of TTF progressively decreased as multiple scans were performed, indicating that the oxidised form has some solubility in water and so is lost from the electrode surface. A second one-electron oxidation process was observed for solid samples of TTF when the potential was extended in the

positive potential direction at approximately +0.6 V vs. Ag/AgCl (Figure 4.1c, dashed line) due to the formation of TTF^{2+} , which is water soluble and so is rapidly lost from the electrode surface.⁶²

Figure 4.1d shows a CV of TTF obtained in CHCl_3 with 0.5 M Bu_4NPF_6 as the supporting electrolyte. The voltammogram displays a one-electron chemically reversible oxidation process at $E_{\text{obs}} = +0.36$ V vs. Ag/AgCl (-0.08 V vs. ferrocene^{0/+}). A second process was observed with $E_{\text{p}}^{\text{ox}} = +0.76$ V vs. Ag/AgCl ($+0.32$ V vs. ferrocene^{0/+}) (Figure 4.1d, dashed line) that showed complicated features similar to those often observed for processes involving adsorption⁶⁰ (the forward and backward traces overlap and sharp peaks are present). It is likely that one-electron oxidation of TTF^{+} leads to the dication (TTF^{2+}) that adsorbs onto the Au electrode, due to the higher charged species being insoluble in the low dielectric constant solvent.

4.2.4 Voltammetry of Au/alkanethiol and Au/alkanethiol/lecithin electrodes

Figure 4.2a (red line) shows a CV obtained of an aqueous solution containing a mixture of 10 mM (each) $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ and $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ in water with 0.5 M KCl as the supporting electrolyte, showing a chemically reversible voltammetric process at $E_{\text{obs}} = +0.25$ V vs. Ag/AgCl due to the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ redox couple. When the Au electrode surface was coated with a monolayer of 1-octadecanethiol, the faradaic current response observed for the voltammetrically induced $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ transformation decreased (Figure 4.2a, black line), due to the alkanethiol layer blocking (or reducing) the electron transfer reactions between the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and the Au surface. It was found that an alkanethiol coating period of 2 hours was sufficiently long to reduce the current observed during

the voltammetric scanning experiments to a constant value that did not decrease further with increased coating time.

Figure 4.1 Cyclic voltammograms obtained at a 3 mm diameter planar Au electrode at $22 (\pm 2) ^\circ\text{C}$ at a scan rate of 100 mV s^{-1} . (a) $2.0 \mu\text{g}$ of solid TCNQ deposited onto the electrode surface with the scan conducted in an aqueous solution containing 0.5 M KCl. (b) 5 mM (satd.) of TCNQ in CHCl_3 with 0.5 M Bu_4NPF_6 . (c) $0.82 \mu\text{g}$ of solid TTF deposited onto the electrode surface with the scan conducted in an aqueous solution containing 0.5 M KCl. (d) 2 mM of TTF in CHCl_3 with 0.5 M Bu_4NPF_6 . The dashed line is the first scan extended over a wider potential range.

1-Octadecanethiol [$\text{CH}_3(\text{CH}_2)_n\text{SH}$, $n = 17$] was used to form the SAM because it has been found that alkyl thiols with long chain lengths ($n > 11$) form densely packed, crystalline-like assemblies, with fully extended alkyl chains.^{47, 49, 63} As the chain length decreases, the structures become disordered resulting in numerous pin-holes between the gold surface and the solution. Nevertheless, the presence of the faradaic current in Figure 4.2b indicates that electron transfer reactions were still occurring between the 10 mM $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and the SAM coated Au surface. The decrease in faradaic and capacitive current between the bare electrode and electrode coated with the SAM (Figure 4.2a) is either due to the presence of a small number of pin holes in the SAM allowing the aqueous solution to reach the electrode surface (thus enabling a diminished number of electron transfer reactions to occur), or due to electron tunneling from specific sites on the electrode surface that have a reduced coverage of the SAM.⁴⁷

When the alkanethiol modified electrode was additionally coated with a multilayer of lecithin, the current observed during voltammetric scanning on the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ containing solution diminished more than the current observed with the alkanethiol monolayer, indicating that the lecithin multilayers were further reducing the electron transfer between the Au electrode and the solution phase species (Figure 4.2b, blue line). A 2- μL aliquot of the lecithin (15 mg mL^{-1}) in chloroform solution was used for the coating procedure because it formed the correct size droplet to completely cover the 3 mm diameter electrode surface (Scheme 4.2c). Once the coated electrode is immersed in the aqueous solution, the lipid layers undergo spontaneous rearrangement and reordering to form multilayers of lipid bilayers with water molecules interleaved between the lipid bilayers (Scheme 4.3).⁶⁴⁻⁶⁷ Since the refined lecithin layers consist of a range of lipid acyl chains (in addition to the phosphatidylcholine shown in Scheme 4.1), the multilamellar phases are likely to be

perfectly aligned and defect free, as reflected by the decrease in faradaic current when the lipid layers are added to the SAM modified electrode in Figure 4.2b.

Figure 4.2 (a) Cyclic voltammograms obtained at a 3 mm diameter planar Au electrode with different coatings in water containing 10 mM (each) of $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ and 0.5 M KCl at $22 (\pm 2) ^\circ\text{C}$ at a scan rate of 100 mV s^{-1} . The starting and finishing potential is $+0.75 \text{ V vs. Ag/AgCl}$. (b) Expansion of the data shown in (a)

Scheme 4.3 Proposed structure of electrode/alkanethiol/lecithin/water interfaces and mode of electron transfer through the interfaces

Reflectance FTIR spectra were obtained of the gold electrode that was coated with a monolayer of the alkanethiol (Figure 4.3a) and with the alkanethiol/lecithin multilayers (Figure 4.3b). The FTIR spectrum of the monolayer of 1-octadecanethiol displayed C-H stretching bands at 2916 cm^{-1} and 2846 cm^{-1} , which have been assigned to the CH_2 ν_a and ν_s modes respectively⁴⁷ (background atmospheric water vapor peaks are present as sharp absorbances in the spectrum). The modes were detected at wavenumber values that indicate that the alkyl chains exist in a crystalline state rather than liquid form (the CH_2 ν_a and ν_s bands in the liquid occur at higher wavenumbers; 2924 cm^{-1} and 2855 cm^{-1} respectively).⁴⁷ In addition to the C-H stretching bands at $\sim 2900\text{ cm}^{-1}$, the spectrum of the alkanethiol/lecithin layer showed strong C=O and P=O absorbances at 1740 cm^{-1} and 1065 cm^{-1} respectively. A strong O-H stretching band was also evident in the alkanethiol/lecithin spectrum at 3400 cm^{-1} due to H_2O molecules between the lecithin layers. The C-H stretching mode in the

alkanethiol/lecithin layer was > 10 times more intense than the same band observed in the spectrum of the alkanethiol monolayer due to the much greater thickness of the lecithin multilayers. Based on the intensity of the C-H stretching bands in the FTIR spectrum of the lecithin layers (Figure 4.3b) relative to the alkanethiol monolayer (Figure 4.3a) it can be estimated that there are on average approximately five lecithin bilayers making up the multilamellar lipid phases on the Au electrode.

Figure 4.3 Reflectance FTIR spectra in air of material deposited onto an Au electrode surface. (a) A monolayer of 1-octadecanethiol. (b) A monolayer of 1-octadecanethiol and multilayers of lecithin

4.2.5 Voltammetry of Au/alkanethiol/lecithin-TCNQ electrodes

Experiments were next conducted by incorporating TCNQ into the lecithin lipid multilayers. This was performed by preparing a saturated solution of TCNQ in chloroform, then adding an aliquot to 500- μL of the lecithin solution, and finally

depositing 2 μL of the TCNQ/lecithin mixture onto the electrode surface and allowing the chloroform to evaporate in air. The amount of lecithin used for coating the electrodes was first determined from electrochemical experiments that were performed by using a constant amount of TCNQ and varying the concentration of lecithin in chloroform between 0.1 – 2% (w/w). It was found that the current observed during the reduction of TCNQ reached a maximum when deposited from a chloroform solution containing between 0.5 – 1% lecithin. Therefore, all subsequent experiments were conducted by depositing TCNQ from a 1% lecithin solution.

The exact location or locations that TCNQ inhabits within the lecithin multilayer structure is currently not known. For example, TCNQ could reside deep within the lecithin bilayers, or reside close to the aqueous solution interface, or be distributed randomly throughout the lecithin multilayer structure. In order for there to be charge neutrality to enable current to flow, the increased negative charge brought about by the reduction process must be balanced by a positive counter-ion (such as potassium) by some means entering the lecithin multilayer structure. Alternatively, $\text{TCNQ}^{\bullet-}$ may be able to momentarily leave the lecithin bilayer during the reduction step (into the aqueous layers separating the bilayers) and then re-enter the lecithin bilayer when it undergoes oxidation back to TCNQ. Due to the large number of uncertainties involved, it becomes very complicated to accurately describe the electrochemical processes that occur. Therefore, the electrochemical results will be discussed in terms of TCNQ and $\text{TCNQ}^{\bullet-}$ residing within a "membrane", where the membrane can be considered to comprise the entire alkanethiol/lecithin multilayer structure (Scheme 4.3).

Figure 4.4 Cyclic voltammograms obtained at a 3 mm diameter planar Au/alkanethiol/lecithin electrode (containing 0.77 μg of TCNQ) at $22 (\pm 2) ^\circ\text{C}$ at a scan rate of 100 mV s^{-1} and immersed in an aqueous solution. The starting and finishing potential is $+0.75 \text{ V vs. Ag/AgCl}$. (a) The aqueous solution containing 0.5 M KCl. The dashed line is the scan extended over a wider potential range. (b) The aqueous solution containing 0.5 M KCl and 10 mM $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$. (c) The aqueous solution containing 0.5 M KCl and 10 mM $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$. The dotted line shows the position of zero current flow

Figure 4.4a (dashed line) shows the cyclic voltammogram that was obtained when 0.77 μg of TCNQ was deposited from a 1% lecithin solution onto the alkanethiol coated Au electrode surface and immersed in an aqueous solution containing 0.5 M KCl. The potential was scanned from +0.75 V to -0.75 V and back to +0.75 V *vs.* Ag/AgCl. The voltammogram in Figure 4.4a shows a reduction process at approximately +0.2 V *vs.* Ag/AgCl, that must be due to the TCNQ within the membrane being reduced to $\text{TCNQ}^{\bullet-}$. The voltammogram obtained under identical conditions but in the absence of TCNQ within the membrane is shown in Figure 4.2 (blue lines), which showed no voltammetric processes. When the scan direction was reversed at -0.75 V *vs.* Ag/AgCl, an oxidation process was detected at close to +0.25 V *vs.* Ag/AgCl but with an oxidative peak current (i_p^{ox}) much greater than the reductive peak current (i_p^{red}). Figure 4.4a (black line) shows the voltammogram that is obtained when the switching potential is reduced, showing that the forward peak is a similar size but the reverse peak is much smaller due to there being less time for the $\text{TCNQ}^{\bullet-}$ to be generated within the membrane.

The results in Figure 4.4a indicate that a small positive current ($\sim +1$ μA) flows at applied potentials more positive than +0.2 V *vs.* Ag/AgCl when the voltammetric scan is first commenced. The likely reason for this is because some of the TCNQ has undergone spontaneous reduction to $\text{TCNQ}^{\bullet-}$ (due to a reaction with another component in the membrane), facilitated by its very low reduction potential. Previous *in situ* EPR experiments on TCNQ in lipid bilayers have shown that it can undergo spontaneous reduction to its anion radical *via* a complex mechanism.^{43, 68} The presence of hydroxide in the aqueous phase is thought to act as the reductant, with other anions influencing the process. Also important is the presence of a positively charged head

group on the lipid bilayer that stabilizes the $\text{TCNQ}^{\bullet-}$, such as the tetraalkylammonium cation in phosphatidylcholine used in this Chapter.⁶⁸

Figure 4.4b (black line) shows the voltammetric results that were obtained when $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ was added to the aqueous solution. In this instance the shape of the voltammogram changed (compared to Figure 4.4a) and ceased to display features typical of a diffusion only controlled process (which show voltammetric peaks), and instead resembled a voltammogram that is observed for a catalytic process, where a sigmoidal current-voltage curve is obtained. The assignment of a catalytic current is supported by the observation that the current on the forward sweep increases beyond what was observed for TCNQ in solutions containing only KCl (compare Figures 4.4a and 4.4b which show maximum reduction currents of $-2 \mu\text{A}$ and $-6 \mu\text{A}$ respectively). It appears that TCNQ within the membrane is voltammetrically reduced to $\text{TCNQ}^{\bullet-}$, which is subsequently oxidised back to TCNQ by the $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ in solution, and the TCNQ is then reduced again to $\text{TCNQ}^{\bullet-}$, etc, so that a catalytic cycle is established as long as a reducing potential is applied to the Au electrode (Scheme 4.4).

Scheme 4.4 Proposed catalytic cycle for the regeneration of TCNQ from $\text{TCNQ}^{\bullet-}$ in the lipid membrane by one-electron oxidation by $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ present in the aqueous solution

An interesting feature of the voltammograms in Figure 4.4b is that the current at the start of the first voltammetric scan is close to 0 μA (at +0.75 V *vs.* Ag/AgCl). This is because the $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ in solution has oxidised any naturally occurring $\text{TCNQ}^{\bullet-}$ in the membrane back to TCNQ. However, after the first scan is commenced, the current on the second and subsequent scans shows an oxidative current at potentials more positive than +0.2 V *vs.* Ag/AgCl. Figure 4.4c shows the voltammetric results that were obtained when $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ was added to the aqueous solution. In this instance a relatively large positive current (+7 μA) was observed as soon as the scan was commenced (at +0.75 V *vs.* Ag/AgCl). The observation of a positive current implies that the $\text{TCNQ}^{\bullet-}$ must exist as a stable species inside the membrane. Therefore, it is likely that as soon as the coated electrode is added to the solution, the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ reduces the TCNQ to $\text{TCNQ}^{\bullet-}$, which subsequently builds up inside the membrane. $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ has previously been used as the aqueous phase reductant for TCNQ that is present at the interface of an immiscible organic solvent (1,2-dichloroethane).⁶⁹⁻⁷¹

Figure 4.5a shows voltammetric results that were obtained when the Au/alkanethiol/lecithin-TCNQ electrode was placed in an aqueous solution containing 10 mM of both $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ and 0.5 M KCl. The voltammograms showed a mixture of the features observed when the two separate $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ solutions were used. For example, (i) the voltammograms appear to show a catalytic limiting current (rather than diffusion controlled peaks), (ii) the reductive current is larger than when there is no $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ or $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ present, and (iii) a positive current flows at the beginning of the potential scan (at +0.75 V *vs.* Ag/AgCl). According to the Nernst equation, an equilibrium should exist between the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and $\text{TCNQ}/\text{TCNQ}^{\bullet-}$, which controls the amount of $\text{TCNQ}^{\bullet-}$ that is detected when the voltammetric scan is first commenced.^{69,72}

The voltammograms in Figure 4.5a show that as the amount of TCNQ was increased, the current values also increased. It was found that the current did not increase when the amount of TCNQ was increased above 0.8 μg , possibly due to saturation of the membrane with TCNQ. Although the measured current was proportional to the amount of TCNQ, the changes occurred over a very narrow range. An increase in the voltammetric current was also observed when the concentrations of $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ in the aqueous phase were increased.

Figure 4.5b shows the results that were obtained when multiple scans were performed, which illustrates that the voltammetric response remains largely unaffected by repetitive scanning and indicating that both the TCNQ and $\text{TCNQ}^{\cdot-}$ are maintained within the membrane structure. The conclusion that the $\text{TCNQ}^{\cdot-}$ is not lost from the membrane is supported by the data obtained in the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ solution (Figure 4.4c) that showed that the concentration of $\text{TCNQ}^{\cdot-}$ builds up when $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ is used as the reductant in the aqueous solution.

Figure 4.5 Cyclic voltammograms obtained at a 3 mm diameter planar Au/alkanethiol/lecithin electrode at $22 (\pm 2) ^\circ\text{C}$ at a scan rate of 100 mV s^{-1} and immersed in an aqueous solution containing 0.5 M KCl, 10 mM $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ and 10 mM $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$. The starting and finishing potential is $+0.75 \text{ V vs. Ag/AgCl}$. (a) Varying amounts of TCNQ within the lecithin layers. (b) 0.58 μg TCNQ within the lecithin layers and a variable number of scans. (c) 0.58 μg TCNQ within the lecithin layers and variable scan rates. (d) 0.58 μg TCNQ within the lecithin layers and the electrode rotated at varying rates

Figure 4.5c shows results that were obtained at varying scan rates, which show that only a relatively small increase in current is observed as the scan rate is increased. At the fastest scan rate, the forward and reverse processes shift apart substantially, which can be attributed to the effects of uncompensated solution resistance. The observation that the reduction current barely changes with increasing scan rate (ν), rather than showing a $\nu^{1/2}$ dependence that is normally observed for a solution phase process, implies that the reduction of TCNQ within the membrane is at steady state and kinetically limited. Faster scan rates to determine heterogeneous rate constants would require the use of microelectrodes to reduce the effects of uncompensated solution resistance.

While the data in Figure 4.5b indicate that the TCNQ/TCNQ $^{\cdot-}$ largely remain in the membrane with repetitive scanning, the results from rotating disk electrode (RDE) experiments indicated that the lecithin layers are lost from the electrode at fast rotation rates. As the rotation rate is increased from 100 revolutions per minute (RPM) to 5000 RPM, the limiting current values progressively decrease (Figure 4.5d). Similarly, multiple scans at a fixed rotation rate result in the limiting current values progressively decreasing, especially at higher RPM.

4.2.6 Voltammetry of Au/alkanethiol/lecithin-TTF electrodes

Voltammetric experiments were performed by incorporating TTF inside the lecithin multilayers in the same way that TCNQ was added. Figure 4.6a shows the CV that was obtained for 0.68 μg of TTF within the membrane when the aqueous electrolyte consisted of 0.5 M KCl. A chemically irreversible oxidation process was detected at approximately +0.4 V vs. Ag/AgCl, whose current values decreased when multiple scans were performed. CVs performed on TTF in chloroform in the presence of 1% lecithin were the same as CVs obtained in the absence of lecithin, indicating that

TTF⁺ does not directly react with lecithin; therefore, the reason for the lack of the reverse peak during the voltammetric experiments on TTF within the membrane is unlikely to be due to chemical reactivity of TTF⁺. Instead it is more probable that TTF⁺ is rapidly lost from the membrane during the potential scanning experiments.

When the voltammetry was conducted in the presence of 10 mM of K₃[Fe^{III}(CN)₆], the oxidative peak that was previously detected for TTF could not be observed (data not shown). The reason for the absence of the oxidation peak for TTF within the membrane is that the [Fe^{III}(CN)₆]³⁻ in solution has already oxidised the TTF, which is subsequently lost from the membrane. A voltammogram of TTF similar to that shown in Figure 4.6a could be detected when a CV was conducted in the presence of K₄[Fe^{II}(CN)₆], because the reducing nature of [Fe^{II}(CN)₆]⁴⁻ keeps the TTF in its uncharged form. However, no voltammetric peaks were detected when the voltammetry was conducted in solutions containing 10 mM of both K₃[Fe^{III}(CN)₆] and K₄[Fe^{II}(CN)₆] (data not shown).

Voltammetric results obtained at varying scan rates between 0.1 – 10 V s⁻¹ are shown in Figure 4.6b. Because there is a large decrease in the oxidative current between the first and second scans, each voltammogram in Figure 4.6b was obtained from the first scan of a freshly coated electrode. In contrast to the variable scan rate data obtained for TCNQ shown Figure 4.5c, the anodic peak current for the oxidation of TTF follows an approximately $\nu^{1/2}$ dependence. The observed dependence is only approximate because some differences (~10 – 20%) were observed between individual experiments at a fixed concentration. Nevertheless, the increase in peak current with increasing scan rate is clearly greater than observed during voltammograms on TCNQ. Furthermore, the anodic peak currents observed during the oxidation of TTF at a scan rate of 0.1 V s⁻¹ were around 5 times greater than observed for the reduction of TCNQ

at the same scan rate. This observation combined with the variable scan rate studies suggests that $\text{TTF}/\text{TTF}^{+\bullet}$ (Figure 4.6b) is considerably more mobile within the membrane than $\text{TCNQ}/\text{TCNQ}^{\bullet-}$ (Figure 4.5c). The voltammograms in Figure 4.6b also show that the voltammetric wave for the oxidation of TTF shifts to more positive potentials as the scan rate is increased, which may be the result of uncompensated solution resistance within the membrane or a slow rate of heterogeneous electron transfer (experiments at microelectrodes are required to accurately differentiate between the possibilities).

Figure 4.6 Cyclic voltammograms obtained at a 3 mm diameter planar Au/alkanethiol/lecithin electrode (containing $0.68 \mu\text{g}$ of TTF) at $22 (\pm 2) ^\circ\text{C}$ at a scan rate of 100 mV s^{-1} and immersed in an aqueous solution containing 0.5 M KCl . The starting and finishing potential is 0 V vs. Ag/AgCl . (a) Varying number of scans. (b) Varying scan rates

4.3 Discussion

There are several complications that need to be addressed when interpreting the voltammetry of TCNQ and TTF within the membrane structure. The primary issue relates to how it is known that it is the analyte molecules (TCNQ and TTF) within the membranes that undergo the redox reactions. There are a number of studies that report electron transfer reactions through lipid bilayers directly deposited on solid electrodes by dropping solutions containing the lipids onto the electrode surfaces (without a supporting tethered layer such as a SAM).^{9-11, 20-23} However, pure metallic or carbon electrode surfaces are not good structures to deposit robust lipid bilayers free of defects.²⁴ Therefore, an alternative interpretation of the results that must always be considered is if the electron transfer reactions are occurring through defects in the lipid layer(s). Experiments were attempted by depositing the lecithin multilayers directly onto freshly cleaned and polished Au and glassy carbon (GC) electrodes, but found that the electrochemical results were poorly reproducible, possibly because the lecithin was poorly adhering to the electrode surface. The electrochemical results that are obtained for small molecules within lipid bilayers on electrode surfaces are expected to be very different than the results obtained for macromolecules, such as proteins and DNA, which can completely span the bilayer structure. In the case of macromolecules, the supported lipid bilayer structure is often used as a means of anchoring the protein in the vicinity of the electrode.^{9-11, 30, 73, 74}

Although the data in Figure 4.2 show that the alkanethiol/lecithin layers reduce the electron transfer reactions between the Au surface and solution phase $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$, the incorporation of TCNQ and TTF into the lecithin layers could in principle lead to defects in the membrane structure (such as pin holes), resulting in direct electron transfer between the Au electrode and the solution phase $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$

$/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ species. The result is especially complicated in the case of TCNQ because its reduction potential coincides very closely to that of the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ redox couple (compare Figures 4.2a and 4.4). Nevertheless, the data presented in this Chapter does support the lecithin layers containing TCNQ and TTF completely covering the electrode surfaces as represented in Scheme 4.3, based on the following observations. First, a voltammetric process at different potentials can be detected for both TCNQ and TTF within the membranes when there is no $\text{K}_3[\text{Fe}^{\text{III}}(\text{CN})_6]$ or $\text{K}_4[\text{Fe}^{\text{II}}(\text{CN})_6]$ in the aqueous phase (Figures 4.4a and 4.6a). Second, the current magnitudes observed for TCNQ within the membranes in the presence and absence of the $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}/[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ species are close. If holes were appearing in the membrane, then the current values would be expected to be very different. Third, the current increases uniformly when increasing amounts of TCNQ are added to the membrane, albeit over a narrow range (Figure 4.5a).

Another possibility is that the TCNQ (or TTF) exists inside pin holes in the initial alkanethiol monolayer that extend between the Au electrode surface and the bulk solution. Some variations ($\sim 10 - 20\%$) were observed in the peak current values between measurements made with identical concentrations of TCNQ or TTF, but this is to be expected due to slight variations in the structures of the films. However, it is more likely that the presence of pin holes would result in very large differences in peak currents, since it is difficult to reproduce defects consistently, which would result in large changes in the measured current values between experiments.

One important question relates to the form that the analyte molecule (TCNQ or TTF) takes when it is deposited from the solution along with the lecithin layers. The cyclic voltammetric responses shown in Figures 4.4, 4.5 and 4.6 are closer to what are expected for a process where a molecule undergoes heterogeneous electron-transfer

within a fluid medium, rather than a solid-state process where (in the case of TCNQ and TTF) the E_p^{red} and E_p^{ox} peaks are very widely spaced (as shown in Figure 4.1a and 4.1c). Therefore, it appears that the TCNQ and TTF exist inside the membranes as individual molecules with the lecithin behaving as a solvent, rather than TCNQ and TTF precipitating as solid microparticles or films.

The peak currents observed when TCNQ or TTF were incorporated into the lecithin layer ($\sim \pm 10 \mu\text{A}$) and when equivalent amounts of TCNQ or TTF were deposited directly on the electrode surface ($\sim \pm 200$ to $\pm 1000 \mu\text{A}$) were substantially different, with the solid state samples giving rise to much greater currents. Therefore, it is probable that not all of the TCNQ and TTF within the membranes undergo oxidation or reduction. Due to the requirement of charge neutrality, it can be proposed that the TCNQ and TTF need to be positioned within the membrane in such a way that enables them to interact directly with a counter-ion during the electron transfer process, otherwise they will not undergo an electrochemical reaction. Alternatively, the diffusion coefficients of TCNQ and TTF (and their associated ionic forms) could be very low within the membrane, which will reduce the measured current values.

4.4 Conclusions

A method has been developed for the construction of robust lipid (lecithin) bilayers containing TCNQ or TTF on alkanethiol modified gold electrodes. The alkanethiol/lecithin layers can be considered to comprise a membrane structure consisting of a tethered alkanethiol layer with multiple lecithin bilayers making up the multilamellar lipid phases. TCNQ and TTF are able to be voltammetrically reduced ($+0.2 \text{ V vs. Ag/AgCl}$) or oxidised ($+0.4 \text{ V vs. Ag/AgCl}$) within the membrane to form $\text{TCNQ}^{\cdot-}$ and $\text{TTF}^{+\cdot}$, respectively. For TCNQ the reduction process is chemically reversible, while for TTF the oxidation process appears to be chemically irreversible.

The results from variable scan rate experiments indicated that TTF/TTF⁺ was more mobile within the membranes than TCNQ/TCNQ⁻.

TCNQ and TTF within the membrane are able to interact with the aqueous phase [Fe^{II}(CN)₆]⁴⁻ and [Fe^{III}(CN)₆]³⁻ molecules respectively and undergo spontaneous redox reactions, indicating that there is a facile mechanism for electron exchange between the membrane bound molecules and aqueous phase species. The ion-transfer mechanism that must occur in order to account for overall charge neutrality within the membranes is complicated, and it is likely that the exact position of the molecules within the membrane is critical to their ability to undergo electron exchange reactions.

References:

1. Svanholm, U.; Bechgaard, K.; Parker, V. D., *J. Am. Chem. Soc.* **1974**, 96, 2409-2413.
2. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
3. Williams, L. L.; Webster, R. D., *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.
4. Schmid, R.; Goebel, F.; Warnecke, A.; Labanhn, A., *J. Chem. Soc., Perkin Trans. 2* **1999**, 1199-1202.
5. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D., *J. Am. Chem. Soc.* **2009**, 131, 1523-1534.
6. Shearer, M. J.; Seghatchian, M. J., *Vitamin K and Vitamin K-Dependent Proteins: Analytical, Physiological and Clinical Aspects*. CRC Press: Boca Raton, FL, 1993.
7. Blankenship, R. E., *Molecular Mechanisms of Photosynthesis*, 1st ed. Blackwell Science: Oxford, 2002.
8. Trumpower, B. L., *Function of Quinones in Energy Conserving Systems*. Academic Press: New York, 1982.
9. Rusling, J. F., *Acc. Chem. Res.* **1998**, 31, 363-369.
10. Armstrong, F. A.; Wilson, G. S., *Electrochim. Acta* **2000**, 45, 2623-2645.
11. Hu, N., *Pure Appl. Chem.* **2001**, 73, 1979-1991.
12. Tien, H. T.; Ottova, A. L., *Electrochim. Acta* **1998**, 43, 3587-3610.
13. Krysinski, P.; Tien, H. T.; Ottova, A., *Biotechnol. Prog.* **1999**, 15, 974-990.
14. Tien, H. T., *Prog. Sur. Sci.* **1985**, 19, 169-274.
15. Tien, H. T., *J. Phys. Chem* **1984**, 88, 3172-3174.
16. Shirai, O.; Yoshida, Y.; Matsui, M.; Maeda, K.; Kihara, S., *Bull. Chem. Soc. Jpn.* **1996**, 69, 3151-3162.

17. Shiba, H.; Maeda, K.; Ichieda, N.; Kasuno, M.; Yoshida, Y.; Shirai, O.; Kihara, S., *J. Electroanal. Chem.* **2003**, 556, 1-11.
18. Wardak, A.; Tien, H. T., *Bioelectrochem. Bioenerg.* **1990**, 24, 1-11.
19. Martynski, T.; Tien, H. T., *Bioelectrochem. Bioenerg.* **1991**, 25, 317-324.
20. Marchal, D.; Boireau, W.; Laval, J. M.; Moiroux, J.; Bourdillon, C., *Biophys. J.* **1997**, 72, 2679-2687.
21. Jiang, D.; Diao, P.; Tong, R.; Gu, D.; Zhong, B., *Bioelectrochem. Bioenerg.* **1998**, 44, 285-288.
22. Wu, Z.; Tang, J.; Cheng, Z.; Yang, X.; Wang, E., *Anal. Chem.* **2000**, 72, 6030-6033.
23. Zhang, H.; Zhang, Z.; Li, J.; Cai, S., *Int. J. Electrochem. Sci* **2007**, 2, 788-796.
24. Groves, J. T.; Boxer, S. G., *Acc. Chem. Res.* **2002**, 35, 149-157.
25. Brink, G.; Schmitt, L.; Tampe, R.; Sackmann, E., *Biochim. Biophys. Acta Rev. Biomembr.* **1994**, 1196, 227-230.
26. William, L. M.; Evans, S. D.; Flynn, T. M.; Marsh, A.; Knowles, P. F.; Bushby, R. J.; Boden, N., *Langmiur* **1997**, 13, 751-757.
27. Cheng, Y.; Boden, N.; Bushby, R. J.; Clarkson, S.; Evans, S. D.; Knowles, P. F.; Marsh, A.; Miles, R. E., *Langmiur* **1998**, 14, 839-844.
28. Jenkins, A. T. A.; Boden, N.; Bushby, R. J.; Evans, S. D.; Knowles, P. F.; Miles, R. E.; Ogier, S. D.; Schonherr, H.; Vancso, G. J., *J. Am. Chem. Soc.* **1999**, 121, 5274-5280.
29. Jenkins, A. T. A.; Bushby, R. J.; Evans, S. D.; Knoll, W.; Offenhausser, A.; Ogier, S. D., *Langmiur* **2002**, 18, 3176-3180.
30. Jeuken, L. J. C.; Connell, S. D.; Henderson, P. J. F.; Gennis, R. B.; Evans, S. D.; Bushby, R. J., *J. Am. Chem. Soc.* **2006**, 128, 1711-1716.

31. Bunjes, N.; Schmidt, E. K.; Jonczyk, A.; Rippmann, F.; Beyer, D.; Ringsdorf, H.; Graber, P.; Knoll, W.; Naumann, R., *Langmiur* **1997**, 13, 6188-6194.
32. Naumann, R.; Schmidt, E. K.; Jonczyk, A.; Fendler, K.; Kadenbach, B.; Liebermann, T.; Offenhausser, A.; Knoll, W., *Biosens. Bioelectron.* **1999**, 14, 651-662.
33. Giess, F.; Friedrich, M. G.; Heberle, J.; Naumann, R. L.; Knoll, W., *Biophys. J.* **2004**, 87, 3213-3220.
34. Schiller, S. M.; Naumann, R.; Lovejoy, K.; Kunz, H.; Knoll, W., *Angew. Chem. Int. Ed.* **2003**, 42, 208-211.
35. Raguse, B.; Braach-Maksvytis, V.; Cornell, B. A.; King, L. G.; Osman, P. D. J.; Pace, R. J.; Wieczrek, L., *Langmiur* **1998**, 14, 648-659.
36. Krishna, G.; Schulte, J.; Cornell, B. A.; Pace, R.; Wieczorek, L.; Osman, P. D., *Langmiur* **2001**, 17, 4858-4866.
37. Sinner, E. K.; Knoll, W., *Curr. Opin. Chem. Biol.* **2001**, 5, 705-711.
38. Tien, H. T.; Lojewska, Z. K., *Biochem. Biophys. Res. Commum.* **1984**, 119, 372-375.
39. Yamada, H.; Shuiku, H.; Matsue, T.; Uchida, I., *J. Phys. Chem* **1993**, 97, 9547-9549.
40. Cheng, Y.; Schiffrin, D. J., *J. Chem. Soc. Faraday Trans.* **1994**, 90, 2517-2523.
41. Wang, H. M.; Wang, E. K.; Tien, H. T.; Ottova, A. L., *Bull. Electrochem.* **1996**, 12, 496-498.
42. Sabo, J.; Ottova, A.; Laputkova, G.; Legin, M.; Vojcikova, L.; Tien, H. T., *Thin Solid Films* **1997**, 306, 112-118.
43. Bender, C. J., *Anal. Chem.* **1997**, 253, 196-200.
44. Kryszinski, P., *Adv. Mater. Opt. Electron.* **1998**, 8, 121-128.

45. Asaka, K.; Ottova, A.; Tien, H. T., *Thin Solid Films* **1999**, 354, 201-207.
46. Diao, P.; Jiang, D. L.; Cui, X. L.; Gu, D. P.; Tong, R. T.; Zhong, B., *Chin. Chem. Lett.* **1999**, 10, 587-590.
47. Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D., *J. Am. Chem. Soc.* **1987**, 109, 3559-3568, and references therein.
48. Diao, P.; Guo, M.; Jiang, D.; Jia, Z.; Gu, D.; Tong, R.; Zhong, B., *Colloids. Surf. A* **2000**, 175, 203-206.
49. Cannes, C.; Kanoufi, F.; Bard, A. J., *Langmiur* **2002**, 18, 8134-8141.
50. Bond, A. M., *Broadening Electrochemical Horizons*. Oxford University Press Inc.: New York, 2002.
51. Bond, A. M.; Fletcher, S.; Marken, F.; Shaw, S. J.; Symons, P. G., *J. Chem. Soc. Faraday Trans.* **1996**, 92, 3925-3933.
52. Bond, A. M.; Fiedler, D. A., *J. Electrochem. Soc.* **1997**, 144, 1566-1574.
53. Bond, A. M.; Fletcher, S.; Symons, P. G., *Analyst* **1998**, 123, 1891-1904.
54. Suarez, M. F.; Bond, A. M.; Compton, R. G., *J. Solid State Electrochem.* **1999**, 4, 24-33.
55. Bartlett, P. N., *J. Electroanal. Chem.* **1991**, 300, 175-189.
56. Mounts, R. D.; Widlund, K.; Gunadi, H.; Perez, J.; Pech, B.; Chambers, J. Q., *J. Electroanal. Chem.* **1992**, 340, 227-239.
57. Evans, C. D.; Chambers, J. Q., *J. Am. Chem. Soc.* **1994**, 116, 11052-11058.
58. Evans, C. D.; Chambers, J. Q., *Chem. Mater.* **1994**, 6, 454-460.
59. Chambers, J. Q.; Scaboo, K.; Evans, C. D., *J. Electrochem. Soc.* **1996**, 143, 3039-3045.
60. Bard, A. J.; Faulkner, L. R., *Electrochemical methods: fundamentals and applications*. John Wiley: New York, 1st edition 1980, 2nd edition 2001.

61. Larsen, H.; Pedersen, S. U.; Pedersen, J. A.; Lund, H., *J. Electroanal. Chem.* **1992**, 331, 971-983.
62. Shaw, S. J.; Marken, F.; Bond, A. M., *Electroanalysis* **1996**, 8, 732-741.
63. Lahann, J.; Mitragotri, S.; Tran, T.-N.; Kaido, H.; Sundaram, J.; Choi, I. S.; Hoffer, S.; Somorjai, G. A.; Langer, R., *Sciences* **2003**, 299, 371-374.
64. Small, D. M., *J. Lipid Res.* **1967**, 8, 551-557.
65. Asher, S. A.; Pershan, P. S., *Biophys. J.* **1979**, 27, 393-422.
66. Lipowsky, R., *Nature* **1991**, 349, 475-481.
67. Du, X.; Whallon, J. H.; Hollingsworth, R. I., *Langmiur* **1998**, 14, 5581-5585.
68. Ahuja, R. C.; Dringenberg, B. J., *Langmiur* **1995**, 11, 1515-1523.
69. Solomon, T.; Bard, A. J., *J. Phys. Chem.* **1995**, 99, 17487-17489.
70. Zhang, J.; Unwin, P. R., *J. Phys. Chem. B* **2000**, 104, 2341-2347.
71. Zhang, J.; Unwin, P. R., *Phys. Chem. Chem. Phys.* **2002**, 4, 3820-3827.
72. Cheng, Y.; Schiffrin, D. J., *J. Chem. Soc. Faraday Trans.* **1994**, 90, 2517-2523.
73. Rusling, J. F.; Nassar, A.-E. F., *J. Am. Chem. Soc.* **1993**, 115, 11891-11897.
74. Nassar, A.-E. F.; Zhang, Z.; Hu, N.; Rusling, J. F.; Kumosinski, T. F., *J. Phys. Chem. B* **1997**, 101, 2224-2231.

Chapter 5

Electrochemistry of α -Tocopherol (Vitamin E) and α -Tocopherol quinone films deposited on electrode surfaces in the presence and absence of lipid multilayers

5.1 Introduction

In this Chapter, the electrochemical properties of α -TOH have been examined in its pure form, by depositing the oil onto an electrode surface and measuring voltammetric responses in buffered aqueous solutions between pH 3 – 13.¹⁻³ There has been a report of the solid state electrochemical properties of α -TOH in aqueous systems on basal plane pyrolytic graphite electrodes,⁴ but the study was performed without the hindsight of the most recent solution phase electrochemical experiments.⁵⁻¹² The α -tocopherol quinone (Scheme 5.1, α -TOQ) model compound ((CH₃) α -TOQ), where the phytyl chain is replaced with a methyl group, was also synthesized and its solid state electrochemistry examined in order to confirm whether there is a direct transformation between the phenol (α -TOH) and quinone (α -TOQ) during potential cycling. A procedure was developed for electrochemically oxidizing films of α -TOH “sandwiched” on the electrode surface between multilayers of phospholipids (lecithin), and the results compared with the data obtained from films directly in contact with the aqueous solutions.

Scheme 5.1 Mechanism for the electrochemical and chemical oxidation of α -tocopherol in organic solvents. The counter-ions for the charged species are the supporting electrolyte ions and the listed potentials are the approximate formal potentials obtained in acetonitrile with 0.2 M Bu_4NPF_6 at 22 ± 2 °C⁵⁻¹³

5.2 Results and Discussion

5.2.1 Preparation of electrodes and electrode coatings

The electrodes were polished consecutively with P400 (35 μm) P1200 (15.3 μm) P2000 (10.3 μm) and P4000 (6.5 μm) grades of SiC paper followed by polishing with 3 μm and 1 μm grit alumina oxide powder on Buehler Ultra-Pad polishing cloths. For experiments with α -TOH and α -TOQ directly in contact with the electrolyte solution, a 2 μL aliquot of a 2 mM solution of the compound in CH_3CN was deposited on the surface of the electrode with a micropipette and the solvent allowed to evaporate in air.

The procedure for preparing α -TOH inside lipid (lecithin) multilayers was based on a literature method.¹⁴ α -TOH (0.01 g) and lecithin (0.09 g) were combined in 2 mL of chloroform in a vial and placed in a vortex mixture for 1 minute (a clear solution was obtained). The chloroform was slowly removed (~20 minutes) with a rotary evaporator and the α -TOH/lecithin mixture dried under vacuum at room temperature (22 ± 2 $^{\circ}\text{C}$) for 2 hours. 4 mL of ultrapure water was added to the α -TOH/lecithin mixture and the slurry heated to 40 $^{\circ}\text{C}$. The slurry was placed in a vortex mixture for 30 minutes, periodically reheating the solution so that it remained at 40 ± 5 $^{\circ}\text{C}$, and then the solution was finally homogenized in an ultrasonic bath at 40 $^{\circ}\text{C}$ for 10 minutes. A 2 μL aliquot of the homogenized α -TOH/lecithin solution (which appeared as a milky white consistency) was deposited on the surface of the electrode with a micropipette and the water allowed to evaporate in air (around 5 minutes), to produce a film containing 45 μg of lecithin and 5 μg of α -TOH. Experiments were also performed using homogenized solutions of pure lecithin (without the α -TOH).

5.2.2 Electrochemistry on bare electrodes

Figure 5.1 shows a series of data that were obtained by depositing α -TOH onto a GC electrode surface and recording cyclic voltammograms in aqueous solutions at several different pH values. The deposited α -TOH can consist of a thin film, as micro-droplets or as a mixture of both.¹⁻³ The red lines in Figure 5.1 are the first scan, the black lines are the intermediate scans and the blue lines are the final scans. In each case the initial potential was -0.25 V vs. Ag/AgCl and the potential was first scanned in the positive potential direction.

At pH 3, an oxidative peak (E_p^{ox}) was detected at approximately $+0.5$ V vs. Ag/AgCl when the scan was first commenced (Figure 5.1, A_1). The peak current for the oxidative process (i_p^{ox}) initially increased on the second scan but progressively decreased with the 3rd and subsequent scans. Concomitantly to the oxidative process (A_1) becoming smaller with multiple scans, a new reductive process (B_1) became evident at approximately -0.2 V vs. Ag/AgCl, with its own reverse oxidative couple at approximately $+0.2$ V vs. Ag/AgCl (B_2). The processes B_1 and B_2 were not evident if the scan was only commenced in the negative potential direction, indicating that they are associated with a product of the initial oxidation step (A_1).

An explanation for the data in Figure 5.1 at pH 3 is that α -TOH is initially oxidised in process A_1 , and reacts to form a new species with a reduction peak (E_p^{red}) at B_1 and with a reverse oxidation peak (E_p^{ox}) at B_2 (i.e. processes B_1 and B_2 represent a chemically reversible redox couple). The reason that process A_1 diminishes with repetitive scanning is that only some of the α -TOH is able to be oxidised in each scan. This is a common observation for non-conducting oils (and solid particle/films) because the oil micro-droplet or film must be able to accommodate an ion from the supporting electrolyte (in this case the buffer) to maintain localized electroneutrality

which results in a structural change.¹⁻³ The reason that peaks B₁ and B₂ increase in intensity with increasing number of scans is because of a build-up of the oxidised product, and because the product does not appear to be able to be reduced back to the starting material during a cyclic voltammetric scan. The formation of the species responsible for the B₁/B₂ processes was evident at scan rates of at least 50 V s⁻¹, indicating that the transformation occurred rapidly.

The cyclic voltammograms recorded at the other pH values can be interpreted in the same way as the data obtained at pH 3, but show some subtle changes to the voltammetric responses. In particular, (i) the voltammograms obtained at pH 5, 7 and 13 show two oxidative processes [A₁ (or A₁') and A₂], (ii) the voltammograms obtained at pH 9 and 11 show only one oxidative process (A₂) on the first scan with complicated overlapping traces, (iii) the E_p^{red} and E_p^{ox} peak separation (ΔE_{pp}) of the B₁/B₂ process varies as the pH is changed, and (iv) the E_p^{red} value for the B₁ process shifts to more negative potentials as the pH is increased. There was some variability from experiment to experiment on the appearance of the first scan and the magnitude of the peak currents, which was likely caused by small differences in the homogeneity of the films/droplets. In each case, the current magnitude for the product peaks (B₁ and B₂) were of a similar level to those observed for the initial oxidation (A₁ or A₂), which indicates that the species responsible for processes B₁/B₂ was formed in high yield from the oxidation of α -TOH (i.e. it is the major product).

Figure 5.1 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2 \text{ }^{\circ}\text{C}$ of micro-droplets/films of $0.86 \text{ } \mu\text{g}$ α -TOH deposited onto a 3 mm diameter GC electrode in aqueous solution at different pH values. Citric acid–phosphate buffer solutions were used for pH 3, 5 and 7, while Britton–Robinson buffer solutions were used for pH 9, 11 and 13

In order to positively identify the species responsible for the B₁/B₂ processes, voltammetric experiments were conducted by depositing the α -tocopherol quinone (Scheme 5.1, α -TOQ) model compound onto the electrode surface and initially scanning in the negative potential direction (Figure 5.2). The voltammograms in Figure 5.2 obtained at different pH values closely match the features (in terms of the absolute peak potentials and ΔE_{pp} -values) of the B₁/B₂ processes observed during potential cycling experiments on α -TOH (compare B₁ and B₂ in Figures 5.1 and 5.2). Therefore, it can be concluded that B₁ and B₂ in Figure 5.1 are also associated with oxidised and reduced forms respectively of α -TOQ. A perfect match in the B₁ and B₂ processes between Figures 5.1 and 5.2 are not expected because the α -TOQ model compound contains a methyl group in place of the phytol chain and so has different solubility and morphology characteristics from the natural compound. The voltammograms in Figure 5.2 show that the peak currents (i_p^{ox} and i_p^{red}) for B₁ and B₂ *decreased* with repetitive scans, while in Figure 5.1 the peak currents for B₁ and B₂ *increased* or remained constant. The reason for the decrease in peak currents observed for B₁ and B₂ processes in Figure 5.2 is most likely due to the reduced form of the model compound having some solubility in water, whereas the natural compound with the long phytol chain would be expected to be less water soluble and remain attached to the electrode.

Figure 5.2 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2^\circ \text{C}$ of micro-droplets/films of $0.47 \text{ }\mu\text{g}$ α -TOQ model compound deposited onto a 3 mm diameter GC electrode in aqueous solution at different pH values. Citric acid–phosphate buffer solutions were used for pH 5 and 7, while Britton–Robinson buffer solutions were used for pH 9 and 13

Further evidence for the formation of α -TOQ as the major product during the oxidation of α -TOH came from reflectance infrared experiments on the material on the surface of the GC electrode. Figure 5.3 shows reflectance-FTIR spectra of (a) α -TOH, (b) the spectra obtained of the product of the oxidation of α -TOH formed by holding the potential constant at +0.9 V vs. Ag/AgCl at pH 7 for 30 minutes, and (c) the α -TOQ model compound. The spectrum in Figure 5.3b represents a mixture of unreacted starting material combined with α -TOQ, which can be identified by the characteristic C=O absorbance at 1642 cm^{-1} . The reason that the spectrum of the starting material remains in Figure 5.3b is because not all of the α -TOH is able to undergo electrochemical oxidation on the electrode surface.

Figure 5.3 Background subtracted reflectance-FTIR spectra of (a) α -TOH, (b) a mixture of α -TOH and α -TOQ obtained by electrolysing α -TOH on a GC electrode surface at pH 7 in citric acid–phosphate buffer, and (c) α -TOQ

Based on the data in Figures 5.1 – 5.3, the major mechanism for the oxidation of α -TOH on electrode surfaces in aqueous solutions can be given in Scheme 5.2. The reaction involves initial $-2e^-/-H^+$ oxidation to form the phenoxonium cation (α -TO⁺) and subsequent hydroxylation/hydrolysis of the phenoxonium cation to form the quinone (Process A₁). Even at low pH values the hydroxylation reaction occurs, most likely because the electrophilic phenoxonium cation reacts quickly with a water molecule in the *para*-position to the carbonyl group (with subsequent loss of a proton). Because the formation of α -TOQ from α -TOH involves breaking of the chromanol ring structure, the reaction is chemically irreversible under mild electrochemical conditions. Phenols are weakly acidic, thus as the pH becomes increasingly basic, α -TOH would be expected to undergo deprotonation to form the phenolate anion (α -TO⁻) which is easier to oxidise than α -TOH.^{13, 15, 16} Therefore, at high pH values the A₁ oxidation process shifts to less positive potentials to become A₁', which is associated with the $-2e^-$ oxidation of α -TO⁻ to form α -TO⁺ (Scheme 5.2).

The earlier study on the electrochemistry of films of α -TOH correctly concluded that the B₁ process was associated with reduction of α -TOQ, although it was argued that the process involved one-electron reduction to form the semiquinone (α -TOQ^{•-}), which further reacted with one proton to form a neutral radical (α -TOQH[•]) (i.e. a reversible one-electron/one-proton process).⁴ Based on what is known about the reductive electrochemistry of quinones in aqueous and non-aqueous solvents,¹⁷⁻¹⁹ the data can alternatively be interpreted as a two-electron/two-proton process, although the lifetimes of the intermediates generated during the reduction will vary as the pH is changed. For pH values < 7, α -TOQ is reduced in a chemically reversible two-electron and two-proton process to form the hydroxy quinone (α -TOQH₂) (Scheme 5.2). The

observed potential (E_{obs}) of the process varies according to the pH because protons are involved in the reduction reaction.¹⁸ Hence, the measured E_{obs} is shifted from the formal potential (E_f^0) according to the Nernst equation;

$$E_{\text{obs}} = E_f^0 + \frac{RT}{nF} \times \ln \frac{[\alpha\text{-TOQ}][\text{H}^+]^2}{[\alpha\text{-TOQH}_2]} \quad (5.1)$$

where n is the number of electrons transferred (2), R is the gas constant (8.3143 J K⁻¹ mol⁻¹), T is the temperature (in K) and F is the Faraday constant (96485 C mol⁻¹). When $[\alpha\text{-TOQ}] = [\alpha\text{-TOQH}_2]$, then $E_{\text{obs}} = E_{1/2}^r$ (the reversible half-wave potential). According to Equation 5.1, at 25 °C the $E_{1/2}^r$ shifts by -59.2 mV per unit increase in pH (providing the E_f^0 does not significantly vary with the change in pH). The data in Figures 5.1 and 5.2 indicate that the reductive peak potential (B_1) does shift more negative with increasing pH, although for a solid state process there are difficulties in exactly estimating the potential of the $E_{1/2}^r$ -value.

As the pH increases above ~7, the semiquinone ($\alpha\text{-TOQ}^{\bullet-}$) and dianion ($\alpha\text{-TOQ}^{2-}$) (Scheme 5.2) have increased lifetimes, thus the acid dissociation constants of their associated protonated forms need to be incorporated into the Nernst equation.¹⁹ Therefore, the relationship between the E_{obs} of the quinone and the proton concentration can be given by Equation 5.2, where K_{a1} and K_{a2} are the acid dissociation constants of $\alpha\text{-TOQH}_2$ and $\alpha\text{-TOQH}^-$ respectively.

$$E_{\text{obs}} = E_f^0 + \frac{RT}{nF} \times \ln \left(1 + \frac{[\text{H}^+]}{K_{a2}} + \frac{[\text{H}^+]^2}{K_{a1}K_{a2}} \right) \quad (5.2)$$

It has been demonstrated that the reason why only one two-electron process is observed during the reduction of quinones at high pH (rather than two separate one-electron processes) is because the semiquinone radical ($\alpha\text{-TOQ}^{\bullet-}$) and dianion ($\alpha\text{-TOQ}^{2-}$)

TOQ²⁻) undergo strong hydrogen bonding interactions with water molecules, which has the effect of shifting the potential of the second reduction process to be close to the potential of the first process.¹⁷⁻¹⁹ Even in organic solvents, when the water content increases beyond a certain point the two processes merge into one, as has been recently demonstrated during the reduction of vitamin K₁, whose structure is similar to that of α -TOQ.¹⁷ Nevertheless, it is possible that the B₁/B₂ processes observed at the highest pH which display relatively low ΔE_{pp} -values (Figures 5.1 and 5.2) may involve the one-electron reduction of the α -TOQ oil to form the semiquinone radical, rather than the two-electron reduction that generally occurs in aqueous systems for solution phase molecules.

The identity of the species responsible for the A₂ process is difficult to ascertain, although the earlier study tentatively proposed that it was associated with oxidation of the spiro-dimer (Scheme 5.1, SD) that could form *via* dimerization of α -TO[•].⁴ While the neutral radical is known to undergo dimerization,²⁰ α -TO[•] is easier to oxidise than the starting material, and so should undergo immediate further one-electron oxidation at the electrode surface to form α -TO⁺ (Scheme 5.1, blue pathway). The best method to produce α -TO[•] electrochemically is to start with the phenolate anion (α -TO⁻) which is considerably easier to oxidise than α -TOH and so the applied potential is low enough to prevent α -TO[•] being further oxidised to α -TO⁺.¹³ However, the electrochemical results at high pH indicated that the quinone was still the favored product (Figure 5.1), which does not support the formation of the spiro-dimer (which would be expected to increase in yield as the pH increases due to more α -TO⁻ being present). An alternative assignment of A₂ could be the one-electron oxidation of the

Process A₁ (pH < ~10)

Process A₁' (pH > ~10)

Process B₁/B₂ (pH < ~7)

Process B₁/B₂ (pH > ~7)

Scheme 5.2 Mechanism for the electrochemical oxidation of films of α -tocopherol and reduction of films of α -tocopherol quinone attached to electrode surfaces in aqueous solution

cation radical ($\alpha\text{-TOH}^{*+}$) that forms *via* initial one-electron oxidation of $\alpha\text{-TOH}$ (Scheme 5.1, blue pathway). $\alpha\text{-TOH}^{*+}$ has been shown to be surprisingly long-lived (i.e. non-acidic) in low dielectric constant solvents,^{5, 10, 11, 21} thus some could conceivably be produced *inside* the oil droplets during the initial oxidation and then

undergo further one-electron oxidation and proton loss to form $\alpha\text{-TO}^+$, which ultimately further reacts to form $\alpha\text{-TOQ}$.

The data obtained from solid state voltammetric experiments conducted on Au and Pt electrode surfaces were not as clear as those obtained on GC when buffered aqueous solutions were used (Figure 5.4). On Au at pH 7, additional processes were present on the forward scan at $> +0.8$ V vs. Ag/AgCl and on the reverse scan at approximately $+0.5$ V vs. Ag/AgCl that were also evident on the bare Au surface (without $\alpha\text{-TOH}$), and the B_2 response was not present. On Pt at pH 7, the oxidation (A_1 and A_2) and reduction (B_1/B_2) processes were not clearly observable, and the hydrogen evolution reaction interfered with the reductive voltammetry at $\text{pH} \leq 7$.^{22,23} When LiClO_4 was used as the supporting electrolyte (instead of the pH buffers), the voltammetric responses observed on Au and Pt were similar to those observed on GC at pH 7. Background voltammograms recorded at different pH values at the different electrodes (Au, GC and Pt) in the absence of films of $\alpha\text{-TOH}$ are provided in Figure 5.5.

It was found that the position and shapes of the peaks in the voltammograms of $\alpha\text{-TOH}$ films on GC electrodes in the citric acid–phosphate buffer solutions at pH 3, 5 and 7 were similar to voltammograms obtained at the same pH values using the Britton–Robinson buffers. Therefore, the different anions present in the citric acid–phosphate and Britton–Robinson buffer solutions did not strongly influence the voltammetric behavior, even though the anions must be incorporated into the $\alpha\text{-TOH}$ films during the oxidation process in order to maintain electroneutrality.

Figure 5.4 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2 \text{ }^{\circ}\text{C}$ of micro-droplets/films of $0.86 \text{ }\mu\text{g}$ α -TOH deposited onto 3 mm diameter Au and Pt electrodes in citric acid–phosphate buffered solution at pH 7

5.2.3 Electrochemistry on electrodes modified with lecithin multilayers

Experiments were performed by incorporating vitamin E inside lecithin multilayers deposited on electrode surfaces. The experiments were performed in two ways. In the first instance, α -TOH was deposited onto the electrode surface (from a CH_3CN solution) and then a multilayer of lecithin from a homogeneous aqueous solution was added on top (see Experimental section). The second method involved adding the homogeneous mixture of combined α -TOH/lecithin in water, and dropping it onto the surface of the electrode with a micropipette.

Figure 5.5 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2^\circ \text{C}$ of 3 mm diameter Au, GC and Pt electrodes in buffered solutions of varying pH. Citric acid–phosphate buffer solutions were used for pH 3 and 7, while Britton–Robinson buffer solutions were used for pH 9 and 13.

Figure 5.6 shows voltammetric data obtained at different pH values of films of α -TOH on GC electrodes that were further coated with multilayers of lecithin. The results were similar to those obtained without lecithin (compare Figure 5.1) and indicated that α -TOH is oxidised to the quinone (process A_1), which can then be reduced at negative potentials (Process B_1). Process A_1 in Figure 5.6 shifted positively compared to process A_1 in Figure 5.1. The formation of the quinone indicates that the phenoxonium cation (α -TO⁺) formed by initial oxidation of α -TOH must be positioned within the multilayers so that it is able to undergo reactions with water. Furthermore, the A_1 and B_1 voltammetric peaks shift to more negative potentials as the pH is increased, similar to the data obtained in the absence of lecithin multilayers. However, the shifts in potential for both the A and B processes were less than observed for the α -TOH films that were directly in contact with the aqueous solutions, indicating that the lecithin layers were reducing the exposure of the oxidised/reduced α -TOH to the H⁺ and OH⁻ in the buffered solutions. The principal difference between the data in Figure 5.1 and Figure 5.6 is that the B_2 process is not clearly detectable for films of α -TOH in the presence of lecithin as the pH increases above pH 7 (Figure 5.6). The lack of the B_2 peak suggests that the reduced form of α -TOQ is unstable in the presence of lecithin at high pH. At pH 7 an additional process was detected on the reverse scan at +0.5 V vs. Ag/AgCl which appears to be associated with process A_1 , possibly indicating some reversibility between α -TOH and an intermediate cationic form (Scheme 5.1, blue pathway).

Figure 5.6 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2 \text{ }^{\circ}\text{C}$ of micro-droplets/films of $0.47 \text{ }\mu\text{g } \alpha\text{-TOH}$ deposited onto a 3 mm diameter GC electrode at different pH values, and with the $\alpha\text{-TOH}$ films coated with a multilayer of lecithin. Citric acid–phosphate buffer solutions were used for pH 3 and 7, while Britton–Robinson buffer solutions were used for pH 9 and 13

Figure 5.7 shows voltammetric data that were obtained when multilayers of homogenized α -TOH/lecithin were added directly to the GC electrode surface. Interestingly, the results were very similar to the voltammograms that were obtained when the lecithin multilayer was added on top of the α -TOH films (compare Figures 5.6 and 5.7), which indicates that the voltammetric mechanism is largely the same between the two methods of depositing films of α -TOH inside lecithin multilayers. One explanation for the close similarity in the results shown in Figures 5.6 and 5.7 is that some of the α -TOH inside the lecithin multilayers leaches out when the α -TOH/lecithin homogenized solution is added to the electrode surface, so the voltammetry is really representative of films of α -TOH on the electrode surface. However, the reverse argument could also occur if layers of α -TOH on the electrode surface “dissolve” into the lecithin multilayers. In either case, the results indicate that voltammetric data from oil-phase molecules within lipid multilayers are similar to data obtained from oil films attached to electrode surfaces (at least for vitamin E).

Voltammetric experiments on biological molecules in their natural environment (such as within lipid bilayers) are difficult to perform because of the low concentrations of the analyte molecules within the bilayer membranes and because of the small size of individual cells. In addition, it is uncertain what type of voltammetric response would be expected from a molecule inside a bilayer. Bilayer membranes do have solvent-like properties, but they are considerably less fluid than a typical organic solvent used for electrochemical experiments.²⁴ Therefore, classical diffusion-controlled cyclic voltammetric responses would not be expected from molecules inside lipid bilayers and it is possible that a solid state type response would be observed due to there being restricted motion of the analyte molecules. There is also the issue of charge neutrality to consider which requires ions to move across the aqueous-bilayer

membrane interface in order to maintain localized electroneutrality in the presence of oxidation or reduction reactions (the redox process will not occur unless there is a simultaneous ion-transfer process to alleviate charge imbalances).

The voltammograms in Figure 5.7 show that as the pH was increased above 7, both the A_1 and A_1' oxidation processes could be simultaneously observed, with A_1' being the major oxidation response at pH 13. One explanation for this behavior is that the α -TOH exists within the lecithin layers in different environments. The α -TOH that is less exposed to hydroxide ions undergoes oxidation at a more positive potential (A_1) to the α -TOH (or α -TO⁻) that is more exposed to the hydroxide ions (A_1').

Experiments were performed by incorporating α -TOH within lecithin multilayers deposited on Au and Pt electrodes. The results obtained on Pt electrodes were not as good as those obtained on GC, because it appeared that the lecithin did not form a uniform coating on the Pt (several background faradaic processes that were also present on Pt in the buffer solution could be detected). Figure 5.8 shows voltammetric data that were obtained when multilayers of homogenized α -TOH/lecithin were added directly to the Au electrode surface (the data were essentially the same when α -TOH was added first and then the lecithin multilayers placed on top). On Au, the voltammetric results were similar to those obtained on GC (compare Figure 5.7 and 5.8). An interesting feature of the voltammograms on Au is that in the absence of α -TOH/lecithin layers, many background processes are detected on gold at the different pH values (see Figure 5.4 and 5.5). For example, at a pure gold surface at pH 3, the background current increases to very high negative values once the potential is

Figure 5.7 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2^\circ\text{C}$ of $5 \mu\text{g}$ of α -TOH incorporated into lecithin multilayers and then deposited onto a 3 mm diameter GC electrode at different pH values. Britton–Robinson buffer solutions were used for all pH values

scanned more negative than $-0.4\text{ V vs. Ag/AgCl}$. Furthermore, at pH values > 7 , pronounced oxidation and reduction (stripping) processes are evident when the potential is scanned first in the positive, and then in the negative potential directions (Figure 5.5). These extra processes are only partially evident on the α -TOH/lecithin coated Au electrode at pH 13, at $+0.6\text{ V vs. Ag/AgCl}$ on the forward scan and $+0.1\text{ V vs. Ag/AgCl}$ on the reverse scan. These results indicate that the lecithin layers provide a uniform coating of the electrode and prevent most supporting electrolyte ions from reaching the electrode surface.

The amount of α -TOH combined with the lecithin was varied between $0.5 - 10\%$ (w/w), and it was found that the peak currents (measured on the fifth scan) for the oxidation and reduction processes varied linearly with the mass of α -TOH, confirming that the α -TOH was homogeneously mixed with the lecithin (Figure 5.9a). Experiments were also performed by incorporating the α -TOH within the lecithin layers on a GC rotating disk electrode at rotation rates up to 4000 revolutions per minute (RPM) (Figure 5.9b). It was found that the voltammetric responses were independent of the rotation rate of the electrode indicating that the species produced during the oxidation reaction remained within the lecithin layers. The rotating disk electrode experiments also indicated that the lecithin/ α -TOH films strongly adhere to the electrode surface and are not easily displaced by stirring the solutions.

Figure 5.8 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} and at $22 \pm 2^\circ \text{C}$ of $5 \text{ }\mu\text{g}$ of α -TOH incorporated into lecithin multilayers and then deposited onto a 3 mm diameter Au electrode at different pH values. Citric acid–phosphate buffer solutions were used for pH 3 and 7, while Britton–Robinson buffer solutions were used for pH 9 and 13

Figure 5.9 Cyclic voltammograms obtained at a scan rate of 0.1 V s^{-1} at $22 \pm 2^\circ \text{C}$ of α -TOH incorporated into lecithin multilayers and then deposited onto a 3 mm diameter GC electrode in citric acid–phosphate buffered solution at pH 7. (a) Different amounts of α -TOH inside the lecithin layers with each cyclic voltammogram from the 5th scan. (b) Rotating disk electrode experiment with 5 μg of α -TOH within the lecithin layers and with the electrode rotated at 2000 RPM

5.3 Conclusions

Films of α -tocopherol deposited on GC electrode surfaces and placed in aqueous solution can be electrochemically oxidised to form α -tocopherol quinone as the major product over a wide pH range (3 – 13). The electrochemical oxidation process is chemically irreversible; once the α -tocopherol quinone is formed, it cannot be

converted back to α -tocopherol by potential cycling. By comparison with data obtained from the α -tocopherol quinone model compound, it was found that α -tocopherol quinone deposited on the electrode surface can be electrochemically reduced in a two-electron/two-proton process to form the α -tocopherol hydroquinone, which is a chemically reversible process over a wide pH range. The results obtained using a GC electrode were more easily interpreted than those obtained at Au and Pt electrodes because the metallic electrodes displayed additional features that originated from interactions with the supporting electrolyte ions, resulting in metal oxide formation.

Electrochemical experiments performed by incorporating α -tocopherol into lecithin multilayers attached to an electrode surface were very similar to those obtained for pure α -tocopherol films. α -tocopherol was oxidised to α -tocopherol quinone within the lecithin multilayers. However, the reduction process of α -tocopherol quinone appeared to be less chemically reversible as the pH was increased above pH 7. Two different methods were used to incorporate α -tocopherol within the lecithin layers, and both methods yielded similar electrochemical results. The results from experiments performed using a Au electrode were similar to those with the GC electrode and indicated that the lecithin layers were effective at blocking the electrode surface from direct interactions with the electrolyte ions, whilst still allowing conductivity (ion-transfer) between the surface bound molecules and the aqueous solution.

References:

1. Bond, A. M., *Broadening Electrochemical Horizons*. Oxford University Press Inc.: New York, 2002.
2. Marken, F.; Webster, R. D.; Bull, S. D.; Davies, S. G., *J. Electroanal. Chem.* **1997**, 437(1-2), 209-218.
3. Banks, C. E.; Davies, T. J.; Evans, R. G.; Hignett, G.; Wain, A. J.; Lawrence, N. S.; Wadhawan, J. D.; Marken, F.; Compton, R. G., *Phys. Chem. Chem. Phys.* **2003**, 5, 4053-4069.
4. Wain, A. J.; Wadhawan, J. D.; France, R. R.; Compton, R. G., *Phys. Chem. Chem. Phys.* **2004**, 6, 836-842.
5. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D., *J. Org. Chem.* **2005**, 70, 10466-10473.
6. Lee, S. B.; Willis, A. C.; Webster, R. D., *J. Am. Chem. Soc.* **2006**, 128, 9332-9333.
7. 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.
8. Peng, H. M.; Webster, R. D., *J. Org. Chem.* **2008**, 73, 2169-2175.
9. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
10. Williams, L. L.; Webster, R. D., *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.
11. Wilson, G. J.; Lin, C. Y.; Webster, R. D., *J. Phys. Chem. B* **2006**, 110, 11540-11548.
12. Yao, W. W.; Peng, H. M.; Webster, R. D., *J. Phys. Chem. B* **2008**, 112, 6847-6855.
13. Webster, R. D., *Electrochem. Commun.* **1999**, 1, 581-584.
14. Lefevre, T.; Picquart, M., *Biospectroscopy* **1996**, 2, 391-403.

15. Hammerich, O.; Svensmark, B., *Organic Electrochemistry*, 3rd ed. Marcel Dekker: New York, 1991.
16. Nanni, E. J.; Jr.; Stallings, M. D.; Sawyer, D. T., *J. Am. Chem. Soc.* **1980**, 102, 4481-4485.
17. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D., *J. Am. Chem. Soc.* **2009**, 131, 1523-1534.
18. Gupta, N.; Linschitz, H., *J. Am. Chem. Soc.* **1997**, 119, 6384-6391.
19. Quan, M.; Sanchez, D.; Wasylkim, M. F.; Smith, D. K., *J. Am. Chem. Soc.* **2007**, 129, 12847-12856.
20. Bowry, V. W.; Ingold, K. U., *J. Org. Chem.* **1995**, 60, 5456-5467.
21. Lehtovuori, P.; Joela, H., *Phys. Chem. Chem. Phys.* **2002**, 4, 1928-1933.
22. Seriani, N.; Mittendorfer, F., *J. Phys.: Condens. Matter* **2008**, 20, 184023-184034
23. Conway, B. E., *Progress in Surface Science* **1995**, 49, 331-452
24. Groves, J. T.; Boxer, S. G., *Acc. Chem. Res.* **2002**, 35, 149-157.

Chapter 6

A Supported Biomembrane for Examining Electron Transfer and Ion Transfer Reactions of Encapsulated Low Molecular Weight Biological Molecules Using Lecithin and Nafion Modified Electrodes

6.1 Introduction

One major issue with interpreting the results from electrochemical studies on s-BLMs is that defect free bilayer membranes will only form on glass substrates, with metallic and carbon surfaces being poor substrates to obtain ideal membrane coverage.¹ Similarly, even with tethered molecules attached to the electrode surface, such as self-assembled layers of alkane thiols, it is highly likely that pin-holes will exist in the layers.^{2, 3} The presence of pin-holes or defects in the SAMs or s-BLMs means that it is often difficult to determine whether the Faradaic current that is observed during voltammetric measurements is due to the electroactive molecules confined inside the membrane layers, or due to electron transfer reactions that occur between the electrode surface and solution phase molecules that reside in the defects (or channels) in the membrane coating.

In order to overcome the uncertainty of the presence of pin-holes in the s-BLM, this Chapter has expanded and modified the techniques described in Chapters 4 and 5 and developed an alternative approach where the biological molecules are first incorporated into liposomes, and then the liposomes are deposited on the surface of a

chemically modified electrode to spontaneously form multilayers. Using this procedure it is not critical for the electrode to be perfectly coated, since any pin-holes or defects will only incorporate the electrolyte ions, whereas the electroactive molecules will remain completely encapsulated within the membrane structure. The molecule α -tocopherol (vitamin E)⁴⁻¹⁷ that exists naturally within lipid bilayer membranes were chosen to examine because it undergoes redox reactions as part of its *modus operandi* and there exists extensive literature reports of the voltammetric properties of this molecule in both organic solvents (Chapter 3)^{5-10, 12, 13, 15, 17-20} and as oils attached to electrodes in aqueous solutions (Chapter 5).^{11, 17, 21, 22}

Scheme 6.1 Structures of membrane forming molecules

6.2 Results and Discussion

6.2.1 Preparation of electrode surfaces

The electrodes were polished consecutively with P400 (35 μm) P1200 (15.3 μm) P2000 (10.3 μm) and P4000 (6.5 μm) grades of SiC paper followed by polishing with 3 μm and 1 μm grit alumina oxide powder on Buehler Ultra-Pad polishing cloths.

The procedure for preparing the vitamin E inside the lipid (lecithin) multilayers was based on literature methods.^{17, 23} The vitamin E (0.01 g) and lecithin (0.09 g) were combined in 2 mL of chloroform in a vial and placed in a vortex mixture for 1 minute (a clear solution was obtained). The chloroform was slowly removed (~20 minutes) with a rotary evaporator and the vitamin/lecithin mixture dried under vacuum at room temperature (22 ± 2 °C) for 2 hours. 4 mL of ultrapure water was added to the vitamin E/lecithin mixture and the slurry heated to 40 °C. The slurry was placed in a vortex mixer for 30 minutes, periodically reheating the solution so that it remained at 40 ± 5 °C, and then the solution was finally homogenized in an ultrasonic bath at 40 °C for 10 minutes and appeared as a milky white consistency.

In order to provide an optimal droplet size to completely coat the electrode, different μL -sized volumes of the solutions that contained the materials for coating the electrodes were used depending on the size of the electrode to be coated. A 1- μL coating drop volume was used for the 1 mm diameter electrodes and a 2- μL coating drop volume was used for the 3 mm diameter electrodes. A 1- μL or 2- μL droplet of the Nafion solution was placed on the electrode surface with a micropipette and the solvent allowed to evaporate in air for 5 minutes. A 1- μL or 2- μL aliquot of the homogenized vitamin E/lecithin solution was deposited on top of the Nafion coated electrode surface and the water allowed to evaporate in air (around 5 minutes), to

produce a multilayer film containing 22.5 – 45 μg of lecithin and 2.5 – 5 μg of the vitamin E. Three further 1- μL or 2- μL droplets of the Nafion solution were deposited on top of the vitamin E/lecithin multilayers, with the solvent allowed to evaporate for 5 minutes after each addition. The optimal coating procedure described above was determined by a number of trial and error experiments.

6.2.2 Preparation of membranes and electrodes for electrochemistry

Previous studies described experiments where TCNQ²⁰ or vitamin E¹⁷ were incorporated inside lecithin layers and deposited on Au or GC electrode surfaces. Refined lecithin was chosen for the lipid bilayers because it is a naturally occurring phospholipid present in plant and animal tissues. Lecithin consists of a range of lipid acyl chain lengths (in addition to the phosphatidylcholine shown in Scheme 6.1) which favors the formation of bilayer membranes that are perfectly aligned and defect free.²⁴⁻
²⁷ Thus, once the homogenized liposome solutions are placed on the electrode surface, they spontaneously form highly ordered multilamellar phases interleaved with layers of solvent molecules. Based on the amount of lecithin deposited on the electrode surface, and comparing previous FTIR measurements of lecithin coated electrodes,²⁰ it can be estimated that the electrodes used in this Chapter contained on average between 5 – 10 lecithin bilayers making up the multilamellar lipid phases on the electrode surface.

Scheme 6.2 Schematic diagram showing proposed structure of electrode|Nafion|lecithin-vitamin|Nafion|water interfaces

In order to improve the voltammetric responses, the electrode coating procedure described above was modified by the addition of a Nafion layer onto the electrode surface, with another Nafion layer deposited on top of the lecithin multilayers, to form a sandwich structure (Scheme 6.2). Nafion is a cation-exchange polymer with a highly acidic sulfonate group (Scheme 6.1). Electrodes coated with Nafion (and other surface confined molecules) have frequently been used to trap or concentrate solution phase species and thereby improve their analytical detection.²⁸⁻³⁰ In this Chapter the Nafion layers are thought to have two functions; (i) to improve the ion conduction between the electrode surface and the lecithin multilayers and, (ii) to provide a readily

exchangeable source of H^+ to molecules within the membranes in order to balance the charge brought about by electrochemical reactions.

6.2.3 Voltammetry of α -tocopherol (α -TOH)

In order to understand the electrochemical behavior of α -TOH inside the lecithin membranes, it is first necessary to discuss the results from solution phase studies. Detailed electrochemical experiments on α -TOH in dry non-aqueous solvents (such as CH_3CN and CH_2Cl_2) have shown that it undergoes a series of electron transfer and proton transfer reactions (Scheme 6.3),^{4, 6-10, 12-14, 16} which are theoretically similar to those observed for most phenols.³¹⁻³³ Initial one-electron oxidation of α -TOH produces the radical cation, $\alpha\text{-TOH}^{+\bullet}$, which quickly loses a proton to form the neutral phenoxyl radical, $\alpha\text{-TO}^\bullet$. The phenoxyl radical is easier to oxidise than the starting phenol (α -TOH) and so is immediately further oxidised at the electrode surface by one-electron to form a diamagnetic cation ($\alpha\text{-TO}^+$) (often called a phenoxonium cation). Overall, the oxidation mechanism converting α -TOH into $\alpha\text{-TO}^+$ occurs in a chemically reversible two-electron one-proton process (an ECE mechanism). However, it is uncertain whether the second electron transfer step occurs heterogeneously at the electrode surface,¹³ or whether it occurs homogeneously *via* a disproportionation mechanism.^{16, 34}

The major difference in the oxidation mechanism of α -TOH compared to most other phenols is that the $\alpha\text{-TO}^+$ intermediate has a substantial lifetime in non-aqueous solvents, compared to the phenoxonium cations of other phenols which are normally only observed as transient species with lifetimes in the order of μs .³⁵⁻⁴¹ The fate of $\alpha\text{-TO}^+$ formed by initial oxidation of α -TOH does depend critically on the moisture content of the solvent. In acetonitrile with a water content $< \sim 0.05$ M (and in the

absence of other nucleophiles), $\alpha\text{-TO}^+$ survives at 25 °C for a sufficiently long time to be able to be oxidised back to the starting material ($\alpha\text{-TOH}$) at a voltammetric scan rate of 100 mV s⁻¹.

The peak-to-peak separation (ΔE_{pp}) between the forward oxidative (E_{p}^{ox}) and reverse reductive ($E_{\text{p}}^{\text{red}}$) peaks observed during cyclic voltammetry experiments is large ($\Delta E_{\text{pp}} \approx 300 - 400$ mV) due to the proton transfer reaction being interspersed between the electron transfer steps which occur at different potentials (Figure 6.1a). The free proton shown in Scheme 6.3 occurs *via* release of H⁺ from the hydroxyl group during the oxidation process, and exists in solution coordinated to either the solvent or to trace water present in the solvent.⁵ It has been assumed in previous studies that the backward homogeneous reaction steps drawn for the first 3 reactions in Scheme 6.3 involve the same proton that is initially released during the oxidation reaction, rather than from water in the solvent acting as a proton source.^{14,16} As increasing amounts of water are added to the solvent, the lifetime of $\alpha\text{-TO}^+$ decreases as it undergoes a reaction to initially form a hemiketal ($\alpha\text{-TOQ(OH)}$),^{9, 42} which then reacts further to form a quinone ($\alpha\text{-TOQ}$) (Scheme 6.3). The conversion of $\alpha\text{-TO}^+$ into $\alpha\text{-TOQ(OH)}$ in Scheme 6.3 is represented as a hydrolysis reaction, where a water molecule acts in a nucleophilic attack in the aromatic ring. However, in basic conditions the reaction can also occur by direct reaction with hydroxide.

When oil droplets of $\alpha\text{-TOH}$ are deposited on electrode surfaces and the electrodes placed in aqueous solutions, voltammetric oxidation of the $\alpha\text{-TOH}$ droplets at a scan rate of 100 mV s⁻¹ results in the immediate detection of the quinone ($\alpha\text{-TOQ}$) in a chemically irreversible process in high yields (Figure 6.1b).^{11, 17, 22} The process is chemically irreversible in the sense that once the $\alpha\text{-TOQ}$ is formed, it cannot be

converted back to α -TOH simply by reversing the applied potential, because the formation of α -TOQ involves breaking the chromanol ring structure in α -TOH (Scheme 6.3). The first oxidative scan (red line) shows a voltammetric process at approximately +0.2 – +1.0 V vs. Ag/AgCl associated with the oxidation of α -TOH (Figure 6.1b, process A) and a reverse reductive process at approximately –0.6 V vs. Ag/AgCl (Figure 6.1b, process B₁). Process B₁ is only detected if the scan is commenced in the positive direction first, therefore; is associated with a product of the initial oxidation reaction. The identity of the species responsible for process B₁ was determined to be the quinone, α -TOQ (Scheme 6.3), by a comparison of the voltammetry of a pure sample attached to the electrode surface.¹⁷

Scheme 6.3 Electrochemical oxidation mechanism for α -TOH in CH_3CN or CH_2Cl_2 .¹⁶ One resonance structure is displayed for each compound. The counter ions for the charged species are the supporting electrolyte, and the " H^+ " ions likely exist coordinated to the organic solvent (or with trace water).⁵

Figure 6.1 Cyclic voltammograms recorded at a scan rate of 0.1 V s^{-1} at $22 \pm 2^\circ \text{C}$. (a) $1 \text{ mM } \alpha\text{-TOH}$ in CH_3CN containing $0.2 \text{ M Bu}_4\text{NPF}_6$ at a 1 mm diameter planar Pt electrode. (b) $1.7 \mu\text{g } \alpha\text{-TOH}$ deposited on the surface of a 3 mm diameter planar GC electrode and placed in a pH 7 buffered solution

Reduction process B_1 can be further divided into two steps (B_1' and B_1''), either of which can occur depending on the pH of the solution. In the presence of acid, $\alpha\text{-TOQ}$ is reduced in a $+2e^-/+2H^+$ process to form the hydroquinone, $\alpha\text{-TOQH}_2$ (process B_1').^{17-19, 43} In the absence of acid (but in the presence of water), the quinone is reduced in one two-electron process to form the hydrogen bonded dianion, $[\alpha\text{-TOQ}(\text{H}_2\text{O})_x]^{2-}$ (process B_1'').^{18, 43} Thus, at low pH, process B_1 consists of a protonation mechanism (B_1'), at high pH it consists of a hydrogen-bonding mechanism (B_1''), while at

intermediate pH values (depending on the pK_a 's of the species that are involved), the reduction mechanism can occur *via* a mixture of both mechanisms (B_1' and B_1'').⁴³ At intermediate pH values it is difficult to determine whether B_1' or B_1'' are occurring (or both simultaneously), since both occur in one two-electron process.⁴³ Regardless of the exact mechanism, once α -TOQ is formed, the electrochemically induced transformations into α -TOQH₂ or $[\alpha\text{-TOQ}(\text{H}_2\text{O})_x]^{2-}$ are fully chemically reversible with both species being oxidised back to α -TOQ *via* process B_2' or B_2'' , respectively (Figure 6.1b). However, once α -TOH has been oxidised to α -TOQ, it cannot be electrochemically regenerated, so the initial oxidation reaction results in the complete loss of α -TOH.

The existing experiments performed on α -TOH have indicated that electrochemical oxidation in the dry organic solvents (CH_3CN or CH_2Cl_2) results in the formation of $\alpha\text{-TO}^+$ as a long-lived species,^{4, 5, 8-10, 13-16} whilst oxidation of α -TOH in the presence of water results in the rapid (and chemically irreversible) formation of α -TOQ.^{11, 17} An important question relates to the potential oxidation pathway of α -TOH in a bilayer membrane environment similar to where it exists biologically, and whether any of the cationic species shown in Scheme 6.3 can have an appreciable lifetime. The hydrophobic nature of a biomembrane could decrease the rate of potential hydrolysis reactions involving $\alpha\text{-TO}^+$, whilst $\alpha\text{-TOH}^{+*}$ is known to be relatively non-acidic (hence long-lived) in low dielectric constant solvents.^{4, 14, 15}

Although the traditional view of vitamin E's main biological function is that of an antioxidant (essentially being destroyed in a sacrificial reaction with free radicals),⁴⁴⁻⁴⁷ there have been studies that have proposed that its main function is as a cell signaling molecule,^{48, 49} and it has been argued that the antioxidant properties are of secondary importance.^{50, 51} Currently there exists no chemical mechanism to account for

vitamin E's non-antioxidant functions (should they occur), although it is possible that they would involve chemical intermediates such as given in Scheme 6.3. Therefore, the voltammetry of α -TOH inside an electrode-supported biomembrane offers a convenient method of initiating the oxidation reaction and examining the products produced *via* the potential-current traces.

6.2.4 Voltammetry of membranes containing α -tocopherol

Figure 6.2a shows the CVs obtained at different pH values when α -TOH was incorporated inside lecithin layers (without Nafion) and then deposited on the GC electrode surface (see Experimental section). A GC electrode is preferred for these experiments because metallic electrodes often display additional voltammetric processes due to the electrolyte ions interacting with the electrode surface *via* holes in the membrane coverage¹⁷ (holes also exist in the membranes on GC but the adsorption processes with electrolyte anions are less pronounced on GC during voltammetric scanning), alternatively due to less metal oxide formation/hydrogen adsorption process on GC. It can be observed in each case that the first scan (red line) shows an oxidative peak (Process A) between 0 – 1 V *vs.* Ag/AgCl. The peak associated with process A either diminishes immediately after the first scan or diminishes with repetitive scans, to be replaced with new peaks (processes B₁ and B₂) which are associated with reduction of α -TOQ and re-oxidation of its reduced form, respectively (Equation 6.3 – 6.6).

It can be observed in Figure 6.2a that there are differences in the voltammetric traces of α -TOH/ α -TOQ inside the lecithin layers depending on the pH. In particular, as the pH increases the B₂" process becomes increasingly difficult to detect. Furthermore, sometimes additional processes were detected, such as marked with an asterisk in the data at pH 7, which have presently not been assigned to a known

reaction. When examining the redox chemistry of species attached to electrode surfaces is not unusual to observe some variability in the voltammograms between similar experiments, because of small changes in the structure of films on the electrode surface.⁵²⁻⁵⁶

Figure 6.2b shows the results that were obtained when the electrode surface was first modified by the addition of a coating of Nafion, then with the lecithin layers containing α -TOH, then with a further coating of Nafion (the sandwich structure given in Scheme 6.2). Background voltammograms of the Nafion|lecithin|Nafion coatings (without α -TOH) displayed no processes within the measured potential window. The Nafion|lecithin- α -TOH|Nafion coating resulted in a more reproducible and simpler voltammetric response than those shown in Figure 6.2a. In particular, processes B₁ and B₂ were more clearly detected at all pH values and at pH 13 the B₁''/B₂'' processes were very closely spaced. At pH 13, an additional oxidative process was observed during the first voltammetric scan that is labeled A'' in Figure 6.2b. Process A'' was also observed at high pH values when films of α -TOH were placed directly on the electrode surface,¹⁷ and it is proposed that it is associated with oxidation of the phenolate anion form of vitamin E (Scheme 6.3, α -TO⁻), that would be expected to form *via* deprotonation of α -TOH (Equation 6.2). Experiments performed at the same pH produced identical results regardless of whether the Britton–Robinson or citric acid–phosphate buffers were used, indicating that the electrolyte anions were not strongly influencing the voltammetric response. Experiments that were performed by sandwiching α -TOH directly between layers of Nafion without lecithin resulted in voltammetric responses that were poorly reproducible and not well-defined, indicating that the Nafion was working in synergy with the lecithin to improve the voltammetric responses.

Figure 6.2 Cyclic voltammograms recorded at a scan rate of 0.1 V s^{-1} of films of α -TOH deposited on a 3 mm diameter planar GC electrode and placed in buffered solutions at $22 \pm 2^\circ \text{C}$. (a) $5 \mu\text{g}$ of α -TOH inside lecithin layers. (b) $5 \mu\text{g}$ of α -TOH inside Nafion|lecithin|Nafion layers. (c) $5 \mu\text{g}$ of α -TOH inside Nafion|lecithin|Nafion layers with the electrode rotated at 5000 RPM

Voltammograms of α -TOQ produced inside the Nafion|lecithin|Nafion layers obtained at all pH values between 3 – 11 were of a similar appearance, with processes B₁ and B₂ occurring at closely related potentials (over the whole pH range) (Figure 6.2b). This is different than what was observed for α -TOQ produced by oxidizing films of α -TOH directly attached to the electrode surface,^{17, 22} where it was noted that process B₁ shifted to more negative pH values as the pH increased (which could be explained by protons involved in the reduction mechanism effecting the reduction potential due to the equilibrium in the Nernst equation). It can be concluded from the data shown in Figure 6.2b that the Nafion provides a self-contained pH environment, meaning that the voltammetry is less affected by the solution phase pH (Nafion is itself strongly acidic due to the sulfonate groups). The self-contained pH is not, however, maintained at the highest pH (13), where it appears the Nafion coating does not prevent hydroxide ions from reaching the α -TOH within the lecithin. In fact, the response obtained at pH 13 for films of Nafion|lecithin- α -TOH|Nafion was very similar to that obtained when α -TOH was attached directly to the electrode surface.^{17, 22}

Figure 6.2c shows the voltammograms obtained when the Nafion|lecithin- α -TOH|Nafion layers were deposited on a rotating disk GC electrode (RDE) and the electrode rotated at 5000 rpm. The similarity between the voltammograms obtained at the stationary electrode (Figure 6.2b) and RDE (Figure 6.2c) demonstrate that the electrode coatings are robust and not easily dislodged from the electrode surface, and that the voltammetric processes that were observed were entirely due to species present within the membranes and not due to solution phase processes (since there is no evidence of hydrodynamic voltammetric responses that would be expected from solution phase species). It was also found for both the stationary electrodes and RDE

measurements, that the voltammetric response became constant after 5 scans were performed. This indicates that the complete conversion of the α -TOH into α -TOQ occurred within 5 scans (or at least the conversion of all molecules that were electroactive), and that the molecules were not leaving the membrane structure, otherwise the current associated with the B₁/B₂ processes would have been expected to diminish with repetitive scanning.

During any voltammetric process, the electron transfer step(s) have to be accompanied by a corresponding ion-transfer process in order to balance the change in charge. However, the lecithin membranes used in this Chapter provide a natural barrier to the ready transfer of ions. Therefore, it would be expected that as the scan rate increased, the effects of resistance would quickly become apparent because of difficulties in the rapid transfer of ions across the membrane|aqueous solution interface. As the voltammetric scan rate was progressively increased, it was found that films of α -TOH-lecithin that were deposited directly on the GC electrode (without Nafion) did show features expected for high resistance or slow electron transfer; that is, the peak separations were widely spaced, as shown in Figure 6.3a at a scan rate of 100 V s⁻¹ at pH 3 and 7. In contrast, Figure 6.3b shows voltammograms that were obtained for Nafion|lecithin- α -TOH|Nafion layers at a scan rate of 100 V s⁻¹ which show clear voltammetric peaks, less affected by resistance effects.

Figure 6.3 Cyclic voltammograms recorded at a scan rate of 100 V s^{-1} of films of α -TOH deposited on a 1 mm diameter planar GC electrode and placed in buffered solutions at $22 \pm 2 \text{ }^\circ\text{C}$. (a) $2.5 \text{ } \mu\text{g}$ of α -TOH inside lecithin layers. (b) $2.5 \text{ } \mu\text{g}$ of α -TOH inside Nafion|lecithin|Nafion layers

The voltammograms in Figure 6.3 were performed at a 1 mm diameter electrode rather than the 3 mm diameter electrode in order to reduce the effects of uncompensated resistance which are particularly pronounced at fast scan rates at macro-sized electrodes.⁵⁷ The reason that the addition of Nafion films to the lecithin- α -TOH layers provides improved electrochemical results can be explained by it providing an improved proton exchange route across the lipid-aqueous solution interface. Scheme 6.2 displays the proposed interaction between Nafion and lecithin, where the Nafion layer is partially interleaved with the hydrophilic portion of the lecithin bilayers, thereby allowing proton exchange with the membrane bound molecules. It has been proposed that vitamin E has its hydrophilic phenolic head positioned close to the aqueous solution interface and with the phytyl chain pointed into the bilayer membrane as drawn in Scheme 6.2,⁵⁸ which would aid potential proton

exchange reactions. It is possible that the electron transfer (and ion transfer) reactions only occur for vitamins positioned close to the membrane-aqueous solution interface.¹³

In addition to achieving more reproducible voltammetric results, an advantage of sandwiching the lecithin- α -TOH multilayers between the Nafion layers is that it allows the faster scan rate experiments to be performed, thereby potentially allowing reactive intermediate species to be detected. Figure 6.4 shows voltammetric data that were obtained using Nafion|lecithin- α -TOH|Nafion layers at pH 7 and pH 13 over a range of scan rates. It was found that the variable scan rate voltammetric results obtained using the Nafion|lecithin- α -TOH|Nafion layers were essentially the same at all pH values between 3 and 11, while the results obtained at pH 13 were substantially different. The voltammograms in Figure 6.4a (at pH 7) show that as the scan rate increases above approximately 1 V s^{-1} , a new reductive process can be detected, which is labeled as C.

For experiments between pH 3 – 11, process C is only observed if the scan is extended in the positive direction first, to oxidise the α -TOH, and therefore, must involve the reduction of a product of the oxidation of α -TOH. It can be observed in Figure 6.4a, that as the scan rate progressively increases, process C becomes relatively larger compared to process B_1' . However, as multiple scans are performed, the current magnitude of process B_1' increases relative to process C, indicating that the species responsible for C is converting into the species responsible for B_1' (α -TOQ). It can also be observed that the difference between the anodic peak current (i_p^{ox}) for the first and second scans for process A, decreases substantially as the scan rate increases, so at the highest scan rates there is relatively little difference between the first and second scans. Therefore, it can be concluded that; (i) the species responsible for process C is an intermediate between α -TOH (process A) and α -TOQ (processes B_1'/B_2'), and (ii),

at faster scan rates the reduction of the species responsible for process C results in the α -TOH being partially regenerated.

Examination of Scheme 6.3 indicates that there are two candidates for process C; either α -TOH^{•+} or α -TO⁺, both of which are able to undergo reduction back to the starting material. Unfortunately at this time, it is not possible to distinguish between the two possibilities, although it is an interesting and unprecedented observation that a phenolic cation radical or phenoxonium ion can be sufficiently long-lived within a membrane in order to be voltammetrically observed at moderate scan rates, and suggests that such species may be able to exist within biological membranes. The voltammetric detection of an intermediate species also leads to the conclusion that the vitamin E inside the lecithin is partially shielded from water. α -TOH^{•+} is extremely acidic in aqueous systems and so should undergo rapid deprotonation,¹³ while α -TO⁺ is very reactive with water.⁸ Nevertheless, the observation of α -TOQ as the major reaction product within the Nafion|lecithin|Nafion layers does indicate that reactions with water are the major reaction pathway over longer time-scale (> ~0.1 s) voltammetric experiments.

Figure 6.4 Cyclic voltammograms of 2.5 μg of α -TOH inside Nafion|lecithin|Nafion layers deposited on a 1 mm diameter planar GC electrode and placed in buffered solutions at $22 \pm 2^\circ\text{C}$

Voltammetric experiments on the lecithin- α -TOH multilayers sandwiched between the Nafion films produced reproducible anodic peak current values for the first scan that were within 10% of each other at identical scan rates. The Randles-Sevcik equation (Equation 6.7) predicts that the peak current (i_p) is proportional to the square root of the scan rate ($\nu^{1/2}$) for a diffusion controlled electrochemically reversible process, where n is the number of electrons transferred, A is the electrode area, c is the concentration, and D is the diffusion coefficient.⁵⁷

$$i_p = 2.69 \times 10^5 n^{3/2} A c D^{1/2} \nu^{1/2} \quad (6.7)$$

Process A corresponds to the two-electron oxidation of α -TOH within the membrane. Plots of i_p^{ox} vs. $\nu^{1/2}$ for the first scan of process A for the data in Figure 6.4 (left hand column) did display a straight line with a Pearson's r-value of 0.9962 (Figure 6.5). The linear relationship between i_p^{ox} and $\nu^{1/2}$ can be interpreted as the oxidation reaction being diffusion controlled, and that the lecithin films have solvent-like properties. However, it is uncertain whether the diffusion controlled response is due to the α -TOH moving within the membrane or due to the ion-transfer processes that must occur across the membrane interface (in order to balance the change in charge).^{53,55,59-63}

Figure 6.5 Plot of the anodic peak current (i_p^{ox}) for process A (first scan) versus the square root of the scan rate ($v^{1/2}$) obtained from cyclic voltammograms of 2.5 μ g of α -TOH inside Nafion|lecithin|Nafion layers deposited on a 1 mm diameter planar GC electrode and placed in pH 7 buffered solution at 22 ± 2 °C

Figure 6.4b shows the variable scan rate CV results that were obtained using Nafion|lecithin- α -TOH|Nafion layers at pH 13. Regardless of the scan rate, at pH 13 there was no evidence of process C, indicating that the phenoxonium ions of α -TOH were not sufficiently shielded within the membrane environment to prevent rapid reactions with hydroxide and formation of α -TOQ. Variable scan rate CV experiments were also performed by depositing α -TOH directly onto the electrode surface so that the films were directly in contact with the aqueous solutions, but the voltammograms showed no evidence of process C (at any pH).

It is clear from the results in Figures 6.1 – 6.4 that clear voltammetric processes can be observed for the oxidation of α -TOH within the lecithin multilayers, especially when sandwiched between the Nafion films. It can be concluded that the Nafion layers are able to facilitate the ion-transfer between the α -TOH inside the lecithin and the Nafion. The increase in positive charge brought about by the oxidation reaction of α -

TOH can mainly be balanced by the loss of protons from the membrane. Lecithin itself has phosphate groups that can act as a counter-ion for any cationic species produced in the membrane (such as $\alpha\text{-TOH}^{+\bullet}$ or $\alpha\text{-TO}^+$), thus it is not necessary for an anion to transfer into the membrane. The transfer of hydrophilic anions into a hydrophobic organic phase correlates with the Gibbs energy of transfer.⁵⁹⁻⁶³ The observation that the voltammetric results are essentially identical over pH 3 – 11 (and were the same regardless of whether the Britton–Robinson buffers or acetic acid–phosphate buffers were used) indicates that it is mainly the protons within the Nafion that are undergoing exchange, with minimal influence from the anions from the supporting electrolyte in the bulk solution.

6.3 Conclusions

This Chapter has demonstrated that it is possible to construct membranes on electrode surfaces that allow CV experiments to be performed over a wide potential range on molecules confined inside the membranes. The method should be applicable to all low molecular weight biological molecules that exist in lipid bilayer membranes. The membranes when constructed of Nafion|lecithin|Nafion layers allow reproducible voltammograms to be obtained at moderately fast scan rates. The voltammetric results indicate that the membranes have solvent like properties that allow both electron transfer and ion transfer processes to occur. In all the experiments that were conducted on vitamin E within the Nafion|lecithin|Nafion layers, there was no evidence that the organic molecule left the membrane. Instead, the balance in charge that was brought about by the reduction and oxidation processes was controlled by the transfer of protons through the Nafion layers. The function of Nafion is to interface with the hydrophilic groups of the bilayer membranes and thereby provide an improved pathway for ion transfer between the membranes and aqueous solutions.

α -tocopherol undergoes a series of electron and proton transfer reactions that can be voltammetrically detected within the membranes. The processes are complicated but can be interpreted based on previous voltammetric experiments of the compounds in non-aqueous solvents^{4-10, 12-19} and where the compounds are attached to the electrodes in aqueous solutions.^{11, 17, 21, 22} As the voltammetric scan rate was increased, there was evidence of an intermediate cationic species (either the radical cation or phenoxonium cation) that could be reversibly reduced back to the starting material. At slower scan rates, the oxidation of vitamin E resulted in the formation of the α -tocopherol quinone, which was stable inside the membrane environment. The voltammetry of α -tocopherol quinone underwent a chemically reversible transformation into the hydroquinone in a $2e^-/2H^+$ process. For bulk solution pH values between 3 – 11, the Nafion layers were able to shield the membranes from the solution phase electrolytes and thereby provide a constant and low pH environment for the molecules within the membranes during the voltammetric experiments. At pH 13, hydroxide ions were able to enter the membrane structure during slow voltammetric scanning experiments, while at faster scan rates ($v > 2 \text{ V s}^{-1}$), the hydroxide was excluded from the membranes.

References:

1. Groves, J. T.; Boxer, S. G., *Acc. Chem. Res.* **2002**, 35, 149-157.
2. Lahann, J.; Mitragotri, S.; Tran, T.-N.; Kaido, H.; Sundaram, J.; Choi, I. S.; Hoffer, S.; Somorjai, G. A.; Langer, R., *Sciences* **2003**, 299, 371-374.
3. Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D., *J. Am. Chem. Soc.* **1987**, 109, 3559-3568, and references therein.
4. Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D., *J. Org. Chem.* **2005**, 70, 10466-10473.
5. Lee, S. B.; Willis, A. C.; Webster, R. D., *J. Am. Chem. Soc.* **2006**, 128, 9332-9333.
6. Nanni, E. J.; Jr.; Stallings, M. D.; Sawyer, D. T., *J. Am. Chem. Soc.* **1980**, 102, 4481-4485.
7. Parker, V. D., *J. Am. Chem. Soc.* **1969**, 91, 5380-5381.
8. 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.
9. Peng, H. M.; Webster, R. D., *J. Org. Chem.* **2008**, 73, 2169-2175.
10. Svanholm, U.; Bechgaard, K.; Parker, V. D., *J. Am. Chem. Soc.* **1974**, 96, 2409-2413.
11. Wain, A. J.; Wadhawan, J. D.; France, R. R.; Compton, R. G., *Phys. Chem. Chem. Phys.* **2004**, 6, 836-842.
12. Webster, R. D., *Electrochem. Commun.* **1999**, 1, 581-584.
13. Webster, R. D., *Acc. Chem. Res.* **2007**, 40, 251-257.
14. Williams, L. L.; Webster, R. D., *J. Am. Chem. Soc.* **2004**, 126, 12441-12450.
15. Wilson, G. J.; Lin, C. Y.; Webster, R. D., *J. Phys. Chem. B* **2006**, 110, 11540-11548.

16. Yao, W. W.; Peng, H. M.; Webster, R. D., *J. Phys. Chem. B* **2008**, 112, 6847-6855.
17. Yao, W. W.; Peng, H. M.; Webster, R. D., *J. Phys. Chem. C* **2009**, 113, 21805-21814.
18. Hui, Y.; Chng, E. L. K.; Chng, C. Y. L.; Poh, H. L.; Webster, R. D., *J. Am. Chem. Soc.* **2009**, 131, 1523-1534.
19. Hui, Y.; Chng, E. L. K.; Chua, L. P.-L.; Liu, W. Z.; Webster, R. D., *Anal. Chem.* **2010**, 82, 1928-1934.
20. Yao, W. W.; Tan, Y. S.; Low, Y. X.; Yuen, J. S. Y.; Lau, C.; Webster, R. D., *J. Phys. Chem. B* **2009**, 113, 15263-15271.
21. Wadhawan, J. D.; Wain, A. J.; Compton, R. G., *ChemPhysChem* **2003**, 4, 1211-1215.
22. Wain, A. J.; Wadhawan, J. D.; Compton, R. G., *ChemPhysChem* **2003**, 4, 974-982.
23. Lefevre, T.; Picquart, M., *Biospectroscopy* **1996**, 2, 391-403.
24. Asher, S. A.; Pershan, P. S., *Biophys. J.* **1979**, 27, 393-422.
25. Du, X.; Whallon, J. H.; Hollingsworth, R. I., *Langmuir* **1998**, 14, 5581-5585.
26. Lipowsky, R., *Nature* **1991**, 349, 475-481.
27. Small, D. M., *J. Lipid Res.* **1967**, 8, 551-557.
28. Downey, T. M.; Nieman, T. A., *Anal. Chem.* **1992**, 64, 261-268.
29. Kawagoe, K. T.; Garris, P. A.; Wiedemann, D. J.; Wightman, R. M., *Neuroscience* **1992**, 51, 55-64.
30. Ugo, P.; Moretto, L. M., *Electroanalysis* **1995**, 7, 1105-1113.
31. Eickhoff, H.; Jung, G.; Rieker, A., *Tetrahedron* **2001**, 57, 353-364.

32. Hammerich, O.; Svensmark, B., *Organic Electrochemistry*, 3rd ed. Marcel Dekker: New York, 1991.
33. Rieker, A.; Beisswenger, R.; Regier, K., *Tetrahedron* **1991**, 47, 645-654.
34. Costentin, C., *Chem. Rev.* **2008**, 108, 2145-2179.
35. Glover, S. A.; Novak, M., *Can. J. Chem.* **2005**, 83, 1372-1381.
36. Niki, E.; Noguchi, N., *Acc. Chem. Res.* **2004**, 37, 45-51.
37. Novak, M.; Brinster, A. M.; Dickhoff, J. N.; Erb, J. M.; Jones, M. P.; Leopold, S. H.; Vollman, A. T.; Wang, Y.-T.; Glover, S. A., *J. Org. Chem.* **2007**, 72, 9954-9962.
38. Novak, M.; Glover, S. A., *J. Am. Chem. Soc.* **2005**, 127, 8090-8097.
39. Novak, M.; Poturalski, M. J.; Johnson, W. L.; Jones, M. P.; Wang, Y.; Glover, S. A., *J. Org. Chem.* **2006**, 71, 3778-3785.
40. 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.
41. Wang, Y.-T.; Wang, J.; Platz, M. S.; Novak, M., *J. Am. Chem. Soc.* **2007**, 129, 14566-14567.
42. Patel, A.; Netscher, T.; Gille, L.; Mersiter, K.; Rosenau, T., *Tetrahedron* **2007**, 63, 5312-5318.
43. Quan, M.; Sanchez, D.; Wasylikim, M. F.; Smith, D. K., *J. Am. Chem. Soc.* **2007**, 129, 12847-12856.
44. Bowry, V. W.; Ingold, K. U., *J. Org. Chem.* **1995**, 60, 5456-5467.
45. Bowry, V. W.; Ingold, K. U., *Acc. Chem. Res.* **1999**, 32, 27-34.
46. Burton, G. W.; Ingold, K. U., *Acc. Chem. Res.* **1986**, 19, 194-201.
47. Traber, M. G.; Atkinson, J., *Free Radical Biol. Med.* **2007**, 43, 4-15.

48. Boscoboinik, D.; Szewczyk, A.; Hensey, C.; Azzi, A., *J. Biol. Chem.* **1991**, 266, 6188-6194.
49. Tasinato, A.; Boscoboinik, D.; Bartoli, G. M.; Maroni, P.; Azzi, A., *Proc. Natl. Acad. Sci. U. S. A.* **1995**, 92, 12190-12194.
50. Azzi, A., *Free Radical Biol. Med.* **2007**, 43, 16-21.
51. Melton, L., *New Sci* **2006**, 191, 40-43.
52. Banks, C. E.; Davies, T. J.; Evans, R. G.; Hignett, G.; Wain, A. J.; Lawrence, N. S.; Wadhawan, J. D.; Marken, F.; Compton, R. G., *Phys. Chem. Chem. Phys.* **2003**, 5, 4053-4069.
53. Dryfe, R. A. W., *Advances in Chemical Physics*. Wiley: New York, 2009; Vol. 141, p 153-215.
54. Marken, F.; Webster, R. D.; Bull, S. D.; Davies, S. G., *J. Electroanal. Chem.* **1997**, 437(1-2), 209-218.
55. Scholz, F.; Schroder, U.; Gulaboski, R., *Electrochemistry of immobilized particles and droplets*. Springer: Berlin, 2005.
56. Tasakorn, P.; Chen, J.; Aoki, K., *J. Electroanal. Chem.* **2002**, 533, 119-126.
57. Bard, A. J.; Faulkner, L. R., *Electrochemical methods: fundamentals and applications*. John Wiley: New York, 1st edition 1980, 2nd edition 2001.
58. Wang, X.; Quinn, P. J., *Mol. Membr. Bio.* **2000**, 17, 143-156.
59. Komorsky-Lovric, S.; Riedl, K.; Gulaboski, R.; Mirceski, V.; Scholz, F., *Langmuir* **2002**, 18, 8000-8005.
60. MacDonald, S. M.; Opallo, M.; Klamt, A.; Eckert, F.; Marken, F., *Phys. Chem. Chem. Phys.* **2008**, 10, 3925-3933.
61. Quentel, F.; Mirceski, V.; L'Her, M.; Mladenov, M.; Scholz, F.; Elleouet, C., *J. Phys. Chem. B* **2005**, 109, 13228-13236.

62. Rayner, D.; Fietkau, N.; Streeter, I.; Marken, F.; Buckley, B. R.; Page, P. C. B.; Campo, J. d.; Mas, R.; Munoz, F. X.; Compton, R. G., *J. Phys. Chem. C* **2007**, 111, 9992-10002.
63. Scholz, F.; Komorsky-Lovric, S.; Lovric, M., *Electrochem. Commun.* **2000**, 2, 112-118.

Chapter 7

Summary

A series of model tocopherol compounds with differing degrees of methyl substitution around the aromatic ring were studied by variable scan rate (0.1- 500 V s⁻¹) cyclic voltammetry experiments in CH₃CN and CH₂Cl₂. On short voltammetric timescales α -TOH is oxidised in a chemically reversible $-2e^-/-H^+$ process to form a phenoxonium cation (α -TO⁺). The equilibrium and rate constants associated with the chemical steps were estimated by digital simulations of the variable scan rate data over a range of temperatures (T = 253-313K). The rate constants for the protonation step are fast which favours the oxidation processes being chemically reversible over fast and slow timescale experiments. Compounds with less methyl substitution around the aromatic ring were more reactive following oxidation and able to undergo hydrolysis reaction to form hemiketals which isomerize into *p*-quinones. The rate constants for the hydrolysis reaction increase with decreasing methylation of the aromatic ring [(CH₃) α -TO⁺ < (CH₃) γ -TO⁺ $\approx$ (CH₃) δ -TO⁺ < (CH₃)H₃-TO⁺]. The relative lifetimes of the phenoxonium cations were compared with theoretical results obtained from molecular orbital calculations. The reason for the longer lifetime in solution for (CH₃) α -TO⁺ than the other phenoxonium cations is because of a complicated mixture of the charge distribution and steric hindrance.

α -Tocopherol films were deposited on the surface of gold, glassy carbon and platinum electrodes and their voltammetric behavior examined in aqueous solutions between pH 3 and 13. The voltammetric mechanism involved α -tocopherol being

oxidised in a $-2e^-/-H^+$ process to form a phenoxonium cation (similar to the experiments in non-aqueous solvents), which underwent rapid reaction with water (or OH^- at $pH > 7$) and rearrangement to form α -tocopherol quinone in a chemically irreversible process. The identity of α -tocopherol quinone was determined by reflectance-FTIR spectroscopy of the product on the glassy carbon electrode surface and from comparison of the voltammetric data obtained with a sample of the α -tocopherol quinone model compound. It was found that α -tocopherol quinone deposited on the electrode surface can be electrochemically reduced in a $+2e^-/+2H^+$ process to form the α -tocopherol hydroquinone, which is chemically reversible process over a wide pH range. The results obtained using a GC electrode were more easily interpreted than those obtained at Au and Pt electrodes because the metallic electrodes displayed additional features that originated from interactions with the supporting electrolyte ions, more to do with oxide formation/hydrogen adsorption.

Electrochemical experiments were also performed by incorporating α -tocopherol into lecithin multilayers deposited onto the electrode surfaces. The results were very similar to those obtained for pure α -tocopherol films. α -tocopherol was oxidised to α -tocopherol quinone within the lecithin multilayers. However, the reduction process of α -tocopherol quinone appeared to be less chemically reversible as the pH was increased above pH 7. Two methods were used to incorporate α -tocopherol within the lecithin multilayers and both methods yielded similar electrochemical results. The results from experiments performed using a Au electrode were similar to those with the GC electrode and indicated that the lecithin layers were effective at blocking the electrode surface from direct interactions with the electrolyte ions, whilst still allowing

conductivity (ion-transfer) between the surface bound molecules and the aqueous solution.

A robust model membrane environment as developed to enable voltammetry experiments to be performed on α -tocopherol incorporated inside artificial lipid bilayer (or multilayer) membranes. The artificial supported membranes were prepared by sandwiching multilayers of lecithin containing α -tocopherol between layers of Nafion that were deposited on the surface of a glassy carbon electrode. The Nafion films acted as a conduit to aid proton transfer across the lecithin solution interface, and thereby balance the charge brought about by the electrochemical reactions. The method should be applicable to all low molecular weight biological molecules that exist in lipid bilayer membranes. The membranes allow reproducible voltammograms to be obtained at moderately fast scan rates.

The coated electrodes were immersed in aqueous solution between pH 3-13. α -Tocopherol underwent multiple electron-transfer and proton-transfer reactions that can be voltammetrically detected within the membranes. When the voltammetric scan rate was increased, the intermediate cationic species (either the radical cation or phenoxonium cation) was detected. This species could be reversibly reduced back to the starting material. At slow scan rates, α -tocopherol was oxidised and formed α -tocopherol quinone was long-lived inside the membrane environment.

A procedure was developed for initiating electron transfer from a gold electrode to a low molecular weight electron acceptor present inside supported lecithin bilayers, followed by further electron transfer to an electron acceptor present in an aqueous solution. The electron acceptors present in the lecithin bilayers and aqueous phase were 7,7,8,8-tetracyanoquinodimethane (TCNQ) and $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$, respectively. A polished planar gold disk electrode was first coated *via* self-assembled procedures with

an alkanethiol monolayer. A phospholipid layer consisting of multiple bilayers of lecithin containing TCNQ was subsequently deposited onto the alkanethiol monolayer. The Au/alkanethiol/lecithin-TCNQ was placed in an aqueous solution containing various amounts of $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ and $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$, with 0.5 M KCl as the supporting electrolyte. In the absence of TCNQ inside the alkanethiol/lecithin layers, only a small background current was observed. When TCNQ was included in the alkanethiol/lecithin layers, the voltammetry showed features typical of a catalytic process, due to the TCNQ being reduced to $\text{TCNQ}^{\bullet-}$ within the lecithin bilayers and then undergoing oxidation back to TCNQ *via* interaction with $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ at the lecithin-aqueous solution interface. The procedures for preparing the alkanethiol/lecithin-TCNQ coatings were optimized in order to obtain the most reproducible voltammetric response. Experiments were also performed using tetrathiafulvalene (TTF) as an electron donor in the lipid bilayer phase.

Publications

1. Yao, W. W.; Peng, H. M.; Webster, R. D.; Gill, P. M. W.
Variable Scan Rate Cyclic Voltammetry and Theoretical Studies on Tocopherol (Vitamin E) Model Compounds
J. Phys. Chem. B **2008**, 112, 6847-6855
2. Peng, H. M.; Choules, B. F.; Yao, W. W.; Zhang, Z.; Webster, R. D.; Gill, P. M. W.
Long-Lived Radical Cations as Model Compounds for the Reactive One-Electron Oxidation Product of Vitamin E
J. Phys. Chem. B **2008**, 112, 10367-10374
3. Yao, W. W.; Tan, Y. S.; Low, Y. X.; Yuen, J. S. Y.; Lau, C.; Webster, R. D.
Voltammetrically Controlled Electron Transfer Reactions from Alkanethiol Modified Gold Electrode Surfaces to Low Molecular Weight Molecules Deposited within Lipid (Lecithin) Bilayers
J. Phys. Chem. B **2009**, 113, 15263-15271
4. Yao, W. W.; Peng, H. M.; Webster, R. D.
Electrochemistry of α -Tocopherol (Vitamin E) and α -Tocopherol Quinone Films Deposited on Electrode Surfaces in the Presence and Absence of Lipid Multilayers
J. Phys. Chem. C **2009**, 113, 21805-21814
5. Yao, W. W.; Lau, C.; Hui, Y.; Poh, H. L.; Webster, R. D.
An Electrode-Supported Biomembrane for Examining Electron Transfer and Ion Transfer Reactions of Encapsulated Low Molecular Weight Biological Molecules
J. Phys. Chem. C **2011**, 115, 2100-2113