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Ubiquinone electrochemistry in analysis and sensing

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Abstract

Ubiquinone (UQ) is a lipophilic compound present in most living organisms, where UQ's interesting but complex electrochemistry serves an important role in the transfer of electrons and protons within and across the mitochondrial membrane. We briefly review the electrochemical characteristics of UQ and its reduced state, ubiquinol, in solution and immobilized on electrodes, together with its application in electrochemical sensing and detection systems, for example, measuring redox status with reference to reactive oxidative species. The importance of the local environment, solvent, electrolyte, organic membrane, and pH, on the electrochemical behavior of UQ, is also discussed. We discuss techniques used for the direct detection of UQ such as liquid chromatography-electrochemistry. Mediated electrochemistry of UQ allows for quantitative measurements of ions, small molecules, and other analytes such as glucose via chemical sensors and biosensors.

KEYWORDS
bioelectrochemistry, coenzyme q10, electrochemistry, review, sensor, ubiquinone

1 | INTRODUCTION

Ubiquinone (UQ) is a naturally occurring redox-active compound that plays a crucial role in energy transduction in mammals and many bacteria. Its role is as an electron carrier within the mitochondrial electron transport chain that drives the synthesis of adenosine triphosphate (ATP), the primary energy carrier of cells. A series of investigations into the substances and mechanisms involved in biological energy conversion led to UQ and its identification in 1940, culminating with its isolation in 1957 by Crane et al.[1] In 1958, Folkers and co-workers discovered the chemical structure of UQ or coenzyme Q_{10}.[2] It contains a 1,4-benzoquinone group together with a hydrocarbon chain of 10 isoprenyl groups (Figure 1). It is referred to as UQ because of its ubiquitous presence in mammalian and many bacterial cells, and its systematic name is 2-[(2E,6E,10E,14E,18E,22E,26E,30E,34E)-3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaen-1-yl]-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1,4-dione. As a result, it is usually abbreviated to a more convenient name and, in addition to Ubiquinone, it is also widely referred to as Coenzyme Q (CoQ), CoQ_{10}, Ubiquinone-Q_{10}, Ubidecarenone, or Vitamin Q_{10}. Its role in biology is as an enzyme cofactor. In addition to the pivotal role it plays in the biological energy transduction process, UQ also provides antioxidant properties, helping to prevent damage from oxidative stress and other biological processes such as inflammatory responses. The hydrocarbon chain of UQ makes it highly lipid-soluble with a reported log $P > 10$.[3]

In 1975, Mitchell discovered how UQ contributed to ATP synthesis.[4] Mitchell considered the formation of membrane potential as a result of proton gradient generation

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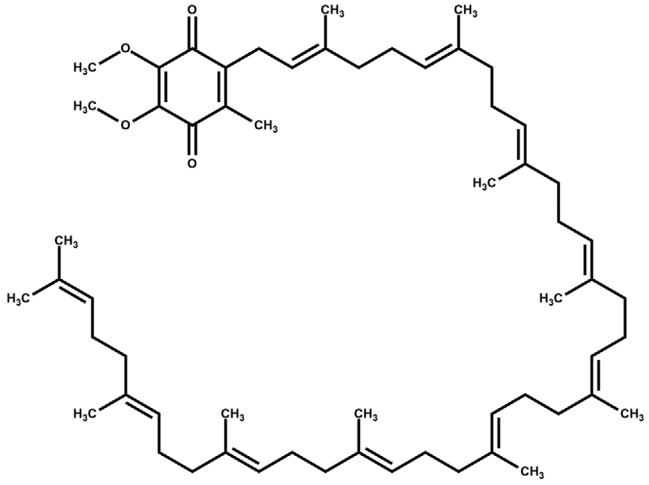

FIGURE 1 Structure of ubiquinone ($C_{59}H_{90}O_4$)

across the membrane which served as the driving force for ATP synthesis via oxidation and reduction of the coenzyme orientated across the membrane. When UQ is reduced, it accepts two protons, which are released when it is oxidized. Therefore, it was recognized that the energy conversion role of UQ was in the protonation and not in the electron transport function. Protons are taken up inside the mitochondrial membrane and released outside as the quinone switches back and forth between its oxidized and reduced forms (Figure 2), leading to the discovery of the unique role of UQ in energy conversion.

There are three redox states of UQ (Figure 3): fully oxidized (UQ), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). According to this, UQ can accept two electrons, to form ubiquinol, or accept one electron to form the semiquinone, followed by acceptance of an additional electron to form the ubiquinol. This capability serves its biological function, being able to accept/donate single electrons as well as pairs of electrons and hence interact with a variety of biological electron transfer partners. The long hydrocarbon chain of UQ (Figure 1) indicates its existence in predominantly lipidic environments in vivo and its preference for non-aqueous environments in vitro.

From an electrochemical perspective, the structure of UQ (Figure 1) possesses many interesting features, not least of which is the 1,4-benzoquinone moiety that is core to its biological function. As a result, reduction/oxidation of the quinone/quinol functionality (0.045 V vs. SHE)[5] has been widely studied. However, the methoxy groups on the 1,4-benzoquinone as well as the alkene groups in the hydrocarbon chain also offer themselves to electrochemical reactivity, although these have been less studied than the 1,4-benzoquinone. Electrochemistry also can be used to determine directly the concentration of UQ/ubiquinol in biological samples, as an indicator of redox capacity and oxidative stress. Immobilization of UQ at electrode surfaces can enable the determination of the concentration of specific oxidative stress indicators (e.g. hydrogen peroxide) in tissues and the development of specific chemical sensors or biosensor devices. However, due to UQ's low solubility in water as well as sensitivity to light and alkaline media, its direct detection in aqueous media is difficult.[6]

The purpose of this mini-review is to focus on the electroanalytical and sensor/biosensor uses of UQ electrochemistry. Although other quinones are active in charge transport within biological systems (including similar quinones with different hydrocarbon chain lengths), here we focus our attention on UQ only. Accordingly, we briefly summarise the electrochemistry of UQ in solution and

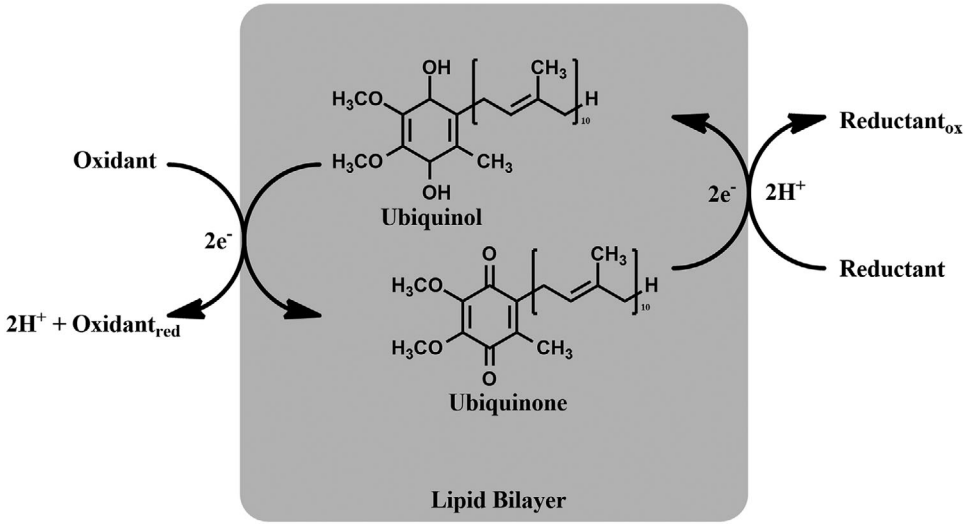

FIGURE 2 Simplified representation of the role of ubiquinone in coupling electron transport to proton transport across bilayer membranes

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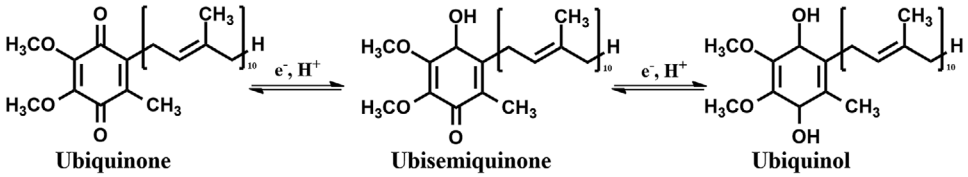

FIGURE 3 Redox states of ubiquinone

modified onto electrode surfaces and then provide a discussion of the applications of UQ electrochemistry, including the determination of UQ in clinical samples, using UQ as a mediator for biosensors, or as a transducer in ion sensor devices.

2 | ELECTROCHEMISTRY OF UQ

In 1961 Moret et al. recorded the electrochemical properties of UQ based on polarographic (i.e. dropping mercury electrode) studies of several biological quinones dissolved in ethanolic aqueous solutions.[7] Since then, the electrochemical behavior of UQ has been further studied using solid electrodes modified with lipid and/or self-assembled hydrocarbon layers, hanging mercury drop electrodes, and an aqueous or non-aqueous medium using a variety of electrochemical techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV).

2.1 | Voltammetry of UQ in solution phase

The electrochemistry of UQ in aqueous conditions is a difficult undertaking due to its hydrophobic nature, stemming from the long isoprenoid hydrocarbon chain on the structure (Figure 1). A study on the dissolution properties of UQ in different solvents by Ondarroa et al. showed UQ to be completely insoluble in water.[8] As a result, modification of the electrode surface and/or the electrolyte solution is required in order to study the redox behavior of UQ in the solution phase. Studies in pure aqueous media at unmodified electrodes are rare. The redox chemistry of UQ can be complex due to the 1,4-benzoquinone moiety having three oxidation states (Figure 3) and each of these states can accept one or two protons, leading to nine different possible redox states, in theory (Figure 4). However, some of these redox states have not been detected experimentally.[9] In a recent thorough review discussing the electrochemical behavior of UQ in solution, Gulaboski et al. discussed the complexity of UQ redox states as well as the possibility of formation of dimers from reactions of UQ

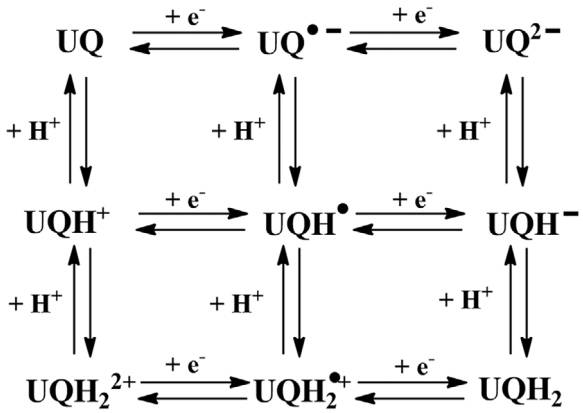

FIGURE 4 Scheme of squares corresponding to the electrochemical-chemical reactions for the ubiquinone (UQ) system.[9,14]

radical species,[10] adding further complexity to UQ electrochemistry. Nevertheless, studies to date on the voltammetric features of UQ have shown that its redox chemistry depends on the electrolyte media, pH, and proton availability. In the excellent review by Gulaboski et al., the key points regarding UQ electrochemistry are summarised. In addition to points mentioned above, these include that UQ electrochemistry in aqueous media is difficult due to the poor solubility of UQ, electrochemistry in aprotic organic media produces two single-electron transfers but in protic organic media a single two-electron transfer is reported. It is worth noting that this complex nature is not isolated to UQ but also applies to other quinone species when studied in the solution phase.[11–13] Additionally, a lipidic environment on the electrode surface provides better conditions to observe electrochemistry in aqueous media, and adsorption to the surface of mercury electrodes is similarly beneficial, both points overcoming the limitations of UQ solubility in aqueous media.[10]

As an example of a recent study of UQ electrochemistry in solution, Li et al. investigated the electrochemical reduction of UQ on a silver electrode in different solvent systems comprising ethanol:H_2O, ethanol, hexane:ethanol, acetic acid:acetonitrile, or isopropanol in the presence or absence of oxygen.[15] The investigations showed that DPV can be used to determine concentrations as low as 33 nM in ethanolic solutions and electrochemical impedance spec-

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troscopy (EIS) proved the impact of oxygen on the electrochemical properties of UQ. Essentially, it was found that an irreversible reaction occurs as oxygen quenches the semiquinone radical formed by the one-electron reduction of UQ.

While most studies of UQ electrochemistry focus on the quinone functionality, the oxidation of the methoxy groups on UQ was found to occur at a potential of *ca*. 1.56 V (vs. Ag|AgCl) on carbon electrodes in acetic acid:acetonitrile solutions containing $NaClO_4$ as a supporting electrolyte.[16] It was found that the number of electrons involved was higher for UQ compared to the similar structure lacking the long isoprenoid chain, indicating that oxidation of the isoprenoid chain was also occurring, a phenomenon also observed by Sabatino et al.[17] Using DPV, highly reproducible peak potentials and currents were recorded which could allow the measurement of UQ concentrations.

2.2 | Immobilized systems

A series of different support systems has been used to immobilize UQ on electrode surfaces. Various electrode systems have been employed, including carbon supports, such as mesoporous carbon, carbon nanotubes, and carbon paste using graphite powder, as well as lipid layers and proteins.

As the classical electrode material of choice, the use of mercury electrodes has provided many insights into the redox behavior of UQ, due in part to the adsorption of lipophilic UQ onto the lipophilic surface of mercury. In the works of Gordillo and Schiffrin, the voltammetry of UQ adsorbed onto the surface of a hanging mercury drop electrode in the presence and absence of lipid on the surface was explored.[18,19] The incorporation of UQ within the lipid matrix made the electron transfer reaction highly irreversible (Figure 5a). They reported that both the orientation of the quinone headgroup in relation to the lipid and the pH dependence of the quinone electrochemistry were responsible for the reversibility of the UQ reaction. At pH 9, the greatest peak-peak separation was found, while increased reversibility (lower peak-peak separation) of the process was observed at pH 14 and under acidic conditions. Subsequently, Heise and Scholz demonstrated how the nature of lipids impacts on redox potentials of UQ. In neutral pH conditions, lower redox potentials were observed in the presence of cardiolipin monolayers compared to monolayers of typical cell membrane lipids, thus suggesting the importance of membrane composition on redox potentials.[20] The pH-dependence of peak potentials and peak separations found by Heise and Scholz was similar to those recorded by Gordillo and Schiffrin for UQ in monolayers of the phospholipid di-oleoyl phosphatidylcholine (DOPC).[19] Katz reported on a polarographic study of ubiquinones with different isoprenoid chains.[21] The major conclusion was that the redox potentials were almost identical for all ubiquinones regardless of the length of the isoprenoid chain. The only difference was observed for UQ-0 (without the chain). The re-orientation of UQ on mercury during reduction was also described and further studied on gold electrode surfaces using Fourier transform infrared (FTIR) spectroscopy.[18,22] The FTIR spectra confirmed that reorientation of the adsorbed molecule occurred on reduction when the aromatic ring formed a strong interaction with the gold surface through pi-bonding. Reorientation of the adsorbed quinol and quinone occurred at potentials more negative and positive than electron transfer, respectively, on mercury electrodes. However, the FTIR measurements showed that electron transfer and reorientation occur simultaneously on gold. In a similar direction, the orientation of UQ and its reaction products on silver electrodes was studied by surface-enhanced Raman scattering and angle-resolved X-ray photoelectron spectroscopy.[23] The spectroscopically-determined orientation of the ring structure on the silver surface was potential dependent.

Mercury electrodes provide valuable insight into the electrochemistry of UQ, however, they are limited in the application of UQ as a sensor device, so other electrode materials compatible with technology have been studied. Performing voltammetry on solid electrodes is quite difficult to achieve as it usually requires the presence of a hydrophobic film but, nevertheless, solid electrode studies allow avenues for applications to be pursued. In this respect, solid electrodes modified with UQ were explored by Takehara and Ide. They reported electron transfer studies of UQ on glassy carbon electrodes in 0.1 M KCl aqueous solution. UQ was spin-coated onto the working electrode surface from an acetone solution.[24] With rapid solvent evaporation, this provided a simple approach to the preparation of films of UQ on the electrode surface which was then examined by electrochemistry of the modified electrode immersed in the aqueous electrolyte solution. They describe the effect of increased concentrations of UQ deposited onto the electrode surface: additional reduction and oxidation peaks were seen at deposition solution concentrations above 1 mM. When 1 mM concentrations or less of UQ were dissolved in the modification solution and subsequently deposited, a monolayer of UQ was formed on the electrode surface. CV oxidation and reduction peaks were assigned to inter- and/or intramolecular proton transfer processes (Figure 5b). Above 1 mM UQ, a multilayer matrix was formed and an electron transfer between adjacent UQ molecules within the

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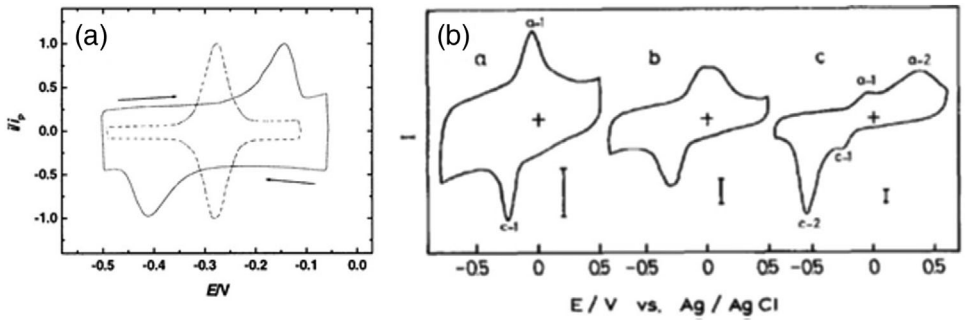

FIGURE 5 (a) Comparative voltammograms (0.1 mV s^{-1}) of ubiquinone (UQ) (ca. 1%) incorporated in di-oleoyl phosphatidylcholine (DOPC) monolayer on a mercury electrode (solid line) and for UQ adsorbed on mercury in the absence of lipid (dashed line), both at pH 9.2. The currents are relative to the maximum peak currents.[19] (b) Cyclic voltammetry (CV, 0.1 mV s^{-1}) of UQ on glassy carbon electrodes in aqueous 0.1 M KCl solution at pH 7.4. The concentration of UQ in the spin-coated solutions was: (a) 0.4, (b) 1.0, and (c) 4.0 mM. Scale bars indicate 2 pA.[24] Part (a) reproduced with permission from *Faraday Discuss.* **2000**, *116*, 89, G.J. Gordillo, D.J. Schiffrin, The electrochemistry of ubiquinone-10 in a phospholipid model membrane, Copyright, The Royal Society of Chemistry **2000**. Part (b) reprinted from *J. Electroanal. Chem*. **1991**, *321*, 297, K. Takehara, Y. Ide, Electrochemical behavior of the ubiquinone-Q10 film coated onto a glassy carbon electrode by the spinner method, Copyright (1991), with permission from Elsevier

inner layer occurred. The surface coverages of UQ in films formed from concentrations of 0.1 and 0.4 mM UQ were 5.1×10^{-11} and 16.3×10^{-11} mol cm^{-2}, respectively, indicating monolayer formation, and in agreement with previously reported values of $1–3 \times 10^{-11}$ mol cm^{-2} for the submonolayer formation of naphthoquinones.[25]

A UQ-based sensor to measure different oxidative stress indicators such as hydrogen peroxide and superoxide was reported by Barsan and Diculescu.[49] In this case, α- or β-cyclodextrins, UQ, and Nafion were employed to form an immobilized UQ film on glassy carbon electrodes. The CV studies of this modified electrode indicated that α-cyclodextrin provided a more favorable electrochemical response, with the peak-peak separation less than that observed for analogous films prepared with β-cyclodextrin. They also observed the effect of the direction of the initial CV scan on the response, where oxidation and reduction peaks were more intense when scanning from a negative to a positive potential. EIS studies also supported the CV results and showed that the α-cyclodextrin-based films were electrochemically superior. Electron microscopic and FTIR spectroscopic studies indicated greater uniformity of the films prepared with α-cyclodextrin, leading to faster electron and ion transfer processes at the membrane/electrode and membrane/solution interfaces, respectively.

Kannan et al. introduced an electrode based on UQ-functionalized mesoporous carbon on glassy carbon for the detection of methyl, ethyl, and propyl parabens in cosmetic products using CV or DPV.[26] The idea here was that UQ serves as an electron mediator that can help to improve the electrochemical behavior of the target compounds, which are redox-active but do not display ideal electrochemical behaviors. The facilitated electron transfer using UQ as a redox mediator resulted in an oxidation peak at 0.76 V versus Ag|AgCl(sat. KCl) that was proportional to the concentration of parabens in cosmetic samples prepared in a pH 7 phosphate buffer solution. A pretreatment electrochemical CV step was performed by scanning from -0.2 to 1.2 V six times prior to measuring parabens in samples. During oxidation of parabens, a redox reaction with ubiquinol (reduced form of UQ) occurs at the film-water interface, similar to previous work by Bilewicz et al. on the mediated-oxidation of reduced ruthenium hexamine complex at UQ modified electrodes.[27]

2.3 | Lipid and self-assembled monolayer films

Lipid films and bilayers and self-assembled monolayers (SAMs) have been important platforms for the study of UQ electrochemistry, not least because these somewhat mimic the natural environment in which UQ functions and hence provide a favorable local environment to probe the electrochemical behavior. For example, the redox feature of lipid membrane-UQ modified electrodes and their coupled electron-transfer reactions with cytochrome P450 (cytP450) in the presence of calcium ions was studied.[28] Thin films of *ca*. 1 μm thickness were prepared by drop-coating a mixture of UQ and DOPC in dichloroethane onto the electrode surface. A specific shift in the UQ/ubiquinol redox potential due to the Ca^{2+} complexation reaction with cytP450 was observed.[28] In the presence of cytP450 or in alkaline conditions, UQ undergoes structural changes whereby one or both of its methoxy groups is demethylated, resulting in the electrochemically reduced forms binding to Ca^{2+} ions.

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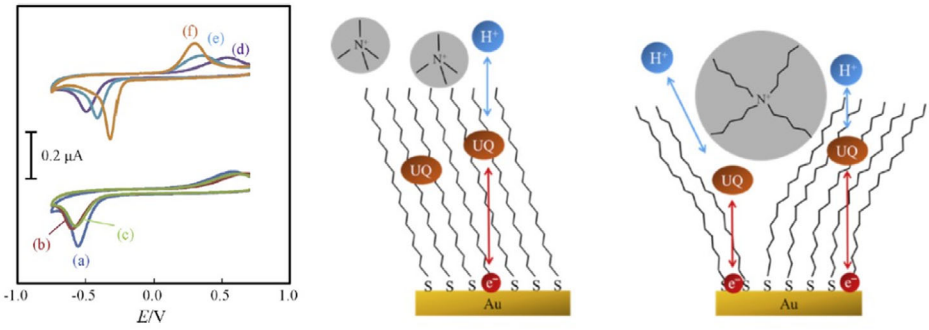

FIGURE 6 Left side: Cyclic voltammetry (CV) of ubiquinone (UQ) in the 1-octyldecylmercaptan (OM) self-assembled monolayer (SAM)-modified gold electrode immersed in aqueous electrolytes of 0.1 M (a) KCl, (b) tetramethylammonium (TMA^+) chloride, (c) tetraethylammonium chloride, (d) tetraproprylammonium chloride, (e) tetrabutylammonium chloride and (f) tetrapentylammonium ($TPnA^+$) chloride (pH 7.2). Scan rate: 0.05 V s^{-1}. Right side: Proposed structural disruption of the SAM induced by the adsorption of hydrophobic supporting-electrolyte cations such as $TPnA^+$ (right) on the SAM surface. A less hydrophobic cation such as TMA^+ (left) does not adsorb on the SAM surface, inducing no disruption of the SAM.[29] Reprinted from *J. Electroanal. Chem*. **2015**, *745*, 22, K. Shiota, M. Ueki, T. Osakai, A role of the membrane|solution interface in electron transfer at self-assembled monolayer modified electrodes, Copyright (2015), with permission from Elsevier

Shiota et al. studied the significance of proton transfer in the redox electrochemistry of UQ.[29] By incorporating UQ within an alkanethiol SAM on a gold electrode, they inhibited the transfer of protons that is normally coupled with UQ's electron transfer reactions. However, they saw that the presence of a hydrophobic cation such as tetrabutylammonium on the surface leads to disruption of the SAM's integrity, allowing movement of protons into the film to react with the UQ (Figure 6). Thus, physical disruption of the SAM by the bulky alkylammonium cation enabled the transfer of protons into the SAM. However, one could also propose that the alkylammonium cations themselves are interacting with the reduced UQ in an ion-binding manner.

Ma et al. described the synthesis of UQ-terminated alkyl disulfides with different alkyl chain lengths and used these to form SAMs that were covalently attached to gold electrode surfaces. During the subsequent formation of a lipid bilayer on the electrode surface, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide hydrogen (NADH) were embedded. As shown in Figure 7, electrochemistry of UQ and its use for mediated electrochemistry of NAD^+/NADH was achieved.[30]

Remarkable stability over several weeks for a UQ-based electrode was achieved by Bilewicz et al. for a coated glassy carbon electrode with a hydrophobin protein, derived from the fungus *Pisolithus tinctorius*, followed by electroreduction of UQ onto the protein.[31] When examined after 3 months of storage at 4°C, the UQ reduction and oxidation peaks grew wider, due to the hydrophobin protein layer undergoing changes leading to the isolation of the electroactive centers of UQ from the electrode surface. Osakai et al. found that a thin film of UQ incorporated as a self-assembled monolayer electrode was able to mediate the reduction of cytochrome c, but not re-oxidation of the reduced protein.[32]

3 | ANALYTICAL APPLICATIONS OF UQ ELECTROCHEMISTRY

In this section, we review the uses of UQ electrochemistry in analytical scenarios, for example in which UQ or its redox-transformation product ubiquinol are detected, or employed as a functional reagent in an analytical device. This direct determination of UQ or ubiquinol or use of the redox properties of the couple as a mediator is reported by various groups. Here the published properties are discussed in terms of direct detection of UQ, use of UQ electrochemistry for detection of other species (ions), and use of UQ electrochemistry in diverse biosensing scenarios.

3.1 | Direct detection of UQ

An important health indicator is a ratio of oxidized to total UQ (i.e., the relative amount of UQ to the total of UQ plus ubiquinol), which has been proposed as a biomarker of oxidative stress. Methods for the detection of UQ and its reduction product, ubiquinol, have been developed and validated by a number of groups. Those employing the electrochemical detection of the target substances following liquid chromatographic (LC) separation have achieved relevant detection limits in biological fluids and these methods are employed in clinical and biomedical research. Bioanalytical methods employing such LC with electrochemical detection (LCEC) methods are also part of a wider repertoire of analytical approaches, together

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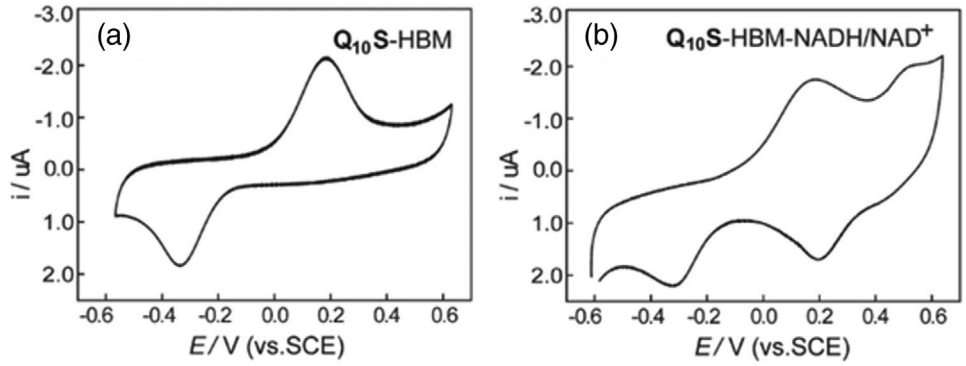

FIGURE 7 Cyclic voltammetry (CVs) at 100 mV s^{-1} of the ubiquinone (UQ)-hybrid bilipid membrane (HBM) system in the absence (a) and presence (b) of embedded nicotinamide adenine dinucleotide hydrogen (NADH)/NAD^+. Reprinted with permission from *J. Am. Chem. Soc.* **2011**, *133*, 12366. Copyright 2011, American Chemical Society

with LC-fluorescence and LC-UV diode array detection for analysis of biological samples for a variety of lipid-soluble antioxidants.[33] The review article by Kalenikova et al. summarises LCEC methods for the quantitation of UQ in biological samples.[34] They state such methods have advantages over other methods, including high selectivity and sensitivity. Franke et al. employed coulometric detection for the determination of UQ/ubiquinol following LC separation, again as biomarkers of redox status and disease risk.[35] Application to blood analysis after suitable sample preparation was achieved, with concentrations found in the range 0.6–3.3 μM, which is substantially higher than the detection limits achievable with LCEC methods. While most LCEC methods for UQ use a coulometric flow cell which offers advantages such as high efficiency in the redox transformation of the target analyte, Dorris and Lunte reported the use of a dual-electrode amperometric flow cell.[36] This cell operated in thin-layer flow mode and enabled a lower potential to be applied for the detection of UQ and ubiquinol. With a limit of detection (LOD) of 5 nM, the method was applied to the determination of UQ and ubiquinol in human plasma. Even earlier, Tang and Miles reported on the measurement of UQ and its reduction product in biofluids precisely for assessing the redox status of individuals.[37] LCEC was used because of its high sensitivity. A dual working electrode flow cell was employed, that enabled reduction of UQ at the first electrode and the oxidation of ubiquinol at the second so that separate analytical signals are obtained for each analyte following the LC separation process. The method can be applied to biofluids as well as cells and tissues, with suitability for clinical laboratories and use in clinical trials.

Recent applications of these LCEC methods include animal samples. Schou-Pedersen et al. employed LCEC for the detection of UQ in canine plasma and heart tissue.[38] The reported lower limit of quantitation was 10 nM, and detection in the plasma in the region of *ca*. 0.6–1.2 μg/ml (ca. 0.7–1.4 μM) was possible for both extracted and non-extracted samples. LC-mass spectrometry (LCMS) methods are also available for such analyses.[39] Similarly, Niklowitz et al. used LCEC to determine UQ and the ratio of UQ/ubiquinol (redox status indicator) whilst also employing coenzyme Q9 (i.e. the quinone moiety has a hydrocarbon chain consisting of nine isoprenyl groups) as an internal standard.[40] The approach was sensitive and detection of the analytes in swine tissues (lung, muscle, brain, liver, etc.) was achieved following a suitable sample preparation method. The wide use of LCEC as the apparent method of choice relative to other LC-detection methods indicates its ideal suitability for the application.

The reliability of the LCEC approach is also reflected in its use in assessing the suitability of other analytical conditions, such as sample storage. In this direction, Matsuo et al. studied the stability of human blood samples taken for UQ/ubiquinol analyses.[41] Since the latter is easily oxidized in air, samples must be stored properly to ensure stability prior to analysis. Using LCEC as the method of choice, they showed that the chemical analysis was consistent for samples stored in a refrigerator or on ice, hence recommending that any storage at room temperature would not produce reliable analytical data. As well as oxidative stress indicators, the use of UQ as an indicator of other diseases is also prevalent. Applications of LCEC methods for UQ/ubiquinol detection include its use in an investigation as to whether oxidative damage of mitochondria was a contributor to Parkinson's Disease.[42] The LCEC detection strategy was a core component in the study that indicated that oxidative damage of mitochondria plays a part in the early stages of Parkinson's Disease development.

While the electrochemical detection of UQ after a chromatographic separation is widely used and reliable, the direct detection without recourse to a prior separation is also worthy of investigation. Direct photoelectrochemical detection of UQ was achieved by Luo et al. using a

dual working electrode approach.[43] At the first electrode, which was glassy carbon, UQ was reduced to ubiquinol. This reduction product was subsequently photo-oxidized at the second electrode, which was indium-doped tin oxide (ITO) coated with a film of ZnO nanorods. The photocurrent on the second electrode exhibited a logarithmic dependence on the UQ concentration and a sub-picomolar LOD was reported. Despite this impressive detection capability, this approach was applied to the determination of UQ in dietary capsules and was not applied to complex samples such as biological materials. Direct electrochemical detection of UQ by reduction at silver electrodes was achieved by Li et al.[15] The reaction was found to depend strongly on the presence of dissolved oxygen in the electrolyte solution. DPV enabled detection over a wide range of concentrations (three orders of magnitude) and a LOD of 33 nM was reported. This approach was applied to the detection of UQ in spiked samples, with excellent recoveries. Likewise, direct detection of UQ on carbon electrodes, specifically glassy carbon and hydrogenated boron-doped diamond (H-BDD), was assessed by Kondo et al. using CV and flow-injection analysis methods.[44] The signal/background ratio was better at the H-BDD electrodes than on glassy carbon. In flow-injection analysis mode, the LOD on the H-BDD electrode was 17 nM, indicating the viable analytical possibilities of this approach. Charoenkitamorn et al. used a modified screen-printed electrode for the direct detection of UQ.[45] A manganese(IV) oxide-modified screen-printed graphene electrode was employed for analysis of drops of sample solutions prepared in an ethanol:water mixture and placed directly onto the screen-printed electrode surface. With a pre-electrolysis step so that UQ was converted to ubiquinol at the initial potential, anodic SWV detection was employed, that is, oxidation of ubiquinol to UQ. The reported LOD was 0.65 μM and the method was applied to the detection of UQ in dietary supplements. The direct determination of UQ by in situ electron paramagnetic resonance (EPR) spectrometry was reported by Long et al.[46] Electrochemical reduction of UQ in ethanol:water mixtures produced a stable radical anion, and the EPR signal provided a LOD of 3 μM.

3.2 | UQ as a mediator or reagent in sensors

3.2.1 | Chemical sensing

The use of UQ as a reagent in the detection of other target substances relies on the use of its redox reactions to manipulate a response to a given target ion. This has been addressed in a number of ways. It relies on two approaches. First, the UQ redox reactions can be used to drive ion binding/ion transfer reactions that in turn enable detection of that ion. Second, UQ can be used as a redox mediator for the detection of small molecules via their reactions with the UQ species attached to an electrode surface.

pH detection with UQ-based electrodes is an obvious possibility given the nature of the coupled electron/proton reactions that UQ undergoes (Figures 2 and 3). McBeth et al. investigated such behavior for the detection of pH in cell culture media.[47] Monitoring pH of cell culture media is an important challenge, not least because conventional pH sensor technology is not amenable to the small volumes and complex sample compositions for such applications. Employing a printed electrode as the substrate, the pH sensor consisted of a mix of carbon nanotubes with Nafion as the electrode surface layer, UQ as the pH-dependent redox probe, and a coating of polyethylene glycol to reduce surface fouling by the biological media. The CV signal of this printed modified electrode indicated an irreversible oxidation peak sensitive to pH in the range of 6–9, which is relevant for cell culture applications. The sensor withstood application in cell culture media with 20% (w/v) serum and was functional after 5 days incubation. pH sensing applications was also investigated by Arthisree et al., who employed a multi-walled carbon nanotube/UQ modified electrode, and found a Nernstian response to pH in the range of 4–13, as well as reactivity with NADH, ascorbic acid, cysteine, hydrogen peroxide, and hydrazine,[48] indicating its possible use in detection of these substances also.

Although UQ and its concentration relative to its reduced form is an indicator of oxidative stress, Barsan et al. employed UQ as a reagent in the detection of other oxidative stress species, specifically hydrogen peroxide and superoxide radical.[49] For this approach, UQ was immobilized on an electrode surface as a complex with alpha-cyclodextrin, together with Nafion (the latter to help ensure film stability). Either constant potential chronoamperometry or SWV was then used for the detection of the target compounds. With this approach, detection at concentrations as low as 0.3 μM was achieved. Interestingly, the fluorescent synthetic analog of UQ designed by Greene et al. was able to detect peroxy radicals, again relevant to the detection of reactive oxygen species.[50]

The detection of parabens, common ingredients in cosmetic products and an important class of emerging environmental pollutants, was investigated by Kannan et al.[26] Using a mesoporous carbon/UQ composite to prepare a modified electrode, the electrocatalytic detection of methyl, ethyl, and propyl parabens was achieved at *ca.* micromolar concentrations. Chokkareddy et al. developed a glassy carbon electrode modified with UQ, iron oxide nanoparticles, and carbon nanotubes, to enable the detection of rifampicin, an antibiotic, achieving a LOD of 0.03 μM.[51]

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The tri-peptide glutathione, an important biomarker of some diseases such as Alzheimer's Disease and Parkinson's Disease, was detected by Ru et al. using an electrode modified with a mixture of UQ, carbon nanotubes, and ionic liquid.[52] With this approach, the reduction current of UQ in the modification film was dependent on the glutathione concentration and enabled a sub-nanomolar LOD. The detection mechanism proposed by Ru et al. involves the electrochemical reduction of glutathione to the disulfide followed by a redox reaction between the disulfide and UQ. Nevertheless, the work of Ru et al. demonstrated that this approach with UQ/carbon nanotubes/ionic liquid film was suitable for the detection of glutathione in blood samples.

Building on the well-studied behavior of UQ at lipid-bilayer modified electrodes, Martensson et al. studied the detection of acetylcholine, which is a non-redox-active neurotransmitter possessing a quaternary ammonium group.[53] Electrochemistry of UQ at a SAM-electrode was used to drive cation transfer via the formation of a tetraalkylammonium-UQ radical anion ion-pair with a resulting shift in the observed UQ redox potential that was dependent on the strength of the ion pair formed. In this way, due to a distinct shift in peak potential, a proposed sensing mechanism was suggested. Although detection limits were not reported, the approach looks interesting and merits further studies to establish sensitivity and selectivity characteristics.

Lawrence et al. also employed UQ within lipid films, in this case on carbon nanoparticles modified with UQ and the lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC).[54] This provided the redox-active carbon particles with the lipid environment that promotes favorable UQ electrochemistry. The electrodes modified with films of these modified carbon particles exhibited well-defined voltammetric responses that were pH-dependent (Figure 8), with evidence that the presence of DMPC on the particle surface introduced some stability. As well as pH dependence, a sodium cation concentration dependence was also seen, suggesting a cation-UQ radical anion ion-pair formation as part of the electrochemical response, similar to the behavior seen by Martensson et al. in lipid layers. Entrapment of UQ in lipid bilayers was also studied by Campos and Kataky, who found that electron transfer in such UQ-containing films was evident, but the addition of the antioxidant vitamin E inhibited the electron transfer.[55]

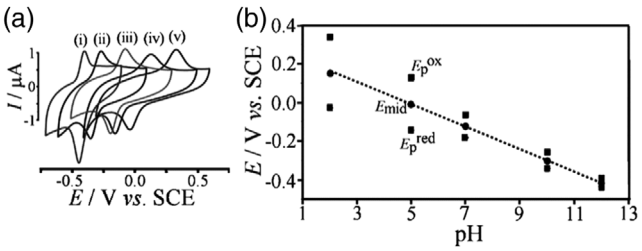

FIGURE 8 (a) Cyclic voltammetry (CV, 10 mV s^{-1}) for the reduction and back-oxidation of ubiquinone (UQ, 0.1 nmol) in 25 nmol 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)/ 15 μg CNP immobilised at a 3 mm diameter glassy carbon electrode and immersed in aqueous 0.5 M phosphate buffer at pH (i) 12, (ii) 10, (iii) 7, (iv) 5, and (v) 2. (b) Plot of oxidation and reduction peak potentials ($E_{p,ox}$ and $E_{p,red}$, respectively) and the midpoint potential E_{mid} (= $[E_{p,ox} + E_{p,red}]/2$) versus pH. Copyright © 2012, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Reproduced with permission from *Electroanalysis* **2012**, *24*, 1003

3.2.2 | Biosensing

The use of UQ electrochemistry in the development and understanding of bioelectrochemistry systems including biosensors has been undertaken by a number of research groups.

UQ operating as a redox mediator within enzyme electrodes was explored by Kawakami et al., who described an enzyme electrode with UQ as a mediator for glucose sensing in phosphate-buffered saline solution.[56] A paste mixture of graphite powder, liquid paraffin, UQ and glucose oxidase was prepared and mounted onto a graphite working electrode.[56] In this study, UQ was assessed for use as a mediator to transfer electrons between the enzyme and the carbon electrode material. From the results presented, mediation with UQ was deemed less efficient compared to other redox mediators such as benzoquinone or ferrocene derivatives. One such reason for this is the presence of the long isoprenoid chain leading to lower diffusivity. Further studies on similar enzyme electrodes by Kawakami et al. investigated the effect of non-ionic surfactants at thin-film oil-water interfaces formed at carbon paste electrodes.[57] They discovered that a thin layer of the surfactant acted as a diffusion barrier on the carbon particles leading to lower current output and increased peak-to-peak separation compared to analogous electrodes prepared without a surfactant present. In another study, the enzymatic activity of cytochrome bo_3, a ubiquinol oxidase in *Escherichia coli* that can oxidize ubiquinol to UQ, was determined by measuring the current produced as a result of the mediated shuttling of electrons from the enzyme by UQ within self-assembled lipid bilayers on Au electrodes.[58,59] This process involved the direct electrochemical reduction of UQ followed by the enzymatic oxidation of the ubiquinol, so producing a current directly related to the enzyme's turnover rate or activity.

The electrochemistry of isolated mitochondria has been investigated to understand the basis of the behavior.

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Giroud et al. studied the electrochemistry of mitochondria from different species immobilized onto carbon electrodes.[60] The electroactivity of the mitochondria was found to be based on UQ rather than other components of the electron transport chain (e.g., the protein cytochrome c). A further study found that mitochondrial electrochemistry could be altered by the addition of effectors.[61] Thus riboflavin derivatives were found to interact with UQ in the mitochondria and to alter the observed electrochemistry. Such electrochemical behavior of these sub-cellular assemblies provides the basis for their use in new biosensing or bioanalytical strategies.

Electrochemistry was employed as a remarkable tool to understand aspects of microbial electrochemistry. Dried films of the bacterium *Shewanella oneidensis* MR-1 were prepared on an ITO electrode.[62] The drying process, with heating, resulted in thermal lysis of the bacterial cells, with the release of UQ which was subsequently detected by voltammetry. With this approach, systematic studies under aerobic and non-aerobic conditions were undertaken and the contribution of UQ to microbial electrochemistry was established. It was demonstrated that this microbial species produced UQ under aerobic conditions and other quinones under anaerobic conditions, but the total quinone content remained constant.

4 | SUMMARY AND OUTLOOK

In this mini-review, we have examined the electrochemical behavior of UQ and its use in analytical and sensing strategies. The overarching characteristic of UQ electrochemistry is its preference for a lipophilic environment, whether in solution (e.g., aqueous media modified with miscible non-aqueous solvents) or on electrode surfaces with or without the presence of lipids. The electrochemistry of UQ is based on transfers of one or two electrons in conjunction with one or two protons, but the interaction of other cationic species opens up means to use UQ electrochemistry in various sensing and detection strategies. The greatest analytical use of UQ electrochemistry is as an electrochemical detector following LC separations for the detection of UQ and ubiquinol in biological samples, which provides an indication of redox status and oxidative stress. The use of UQ as a mediator in bioelectrochemistry has been investigated but the other key analytical applications are in the detection of inorganic ions and small molecules. Future scope for analytical applications of UQ electrochemistry undoubtedly exists, including in biosensing and chemical sensing. Studies in organic thin films[63] on electrodes or at 3-phase junctions[64] should enable extension into new areas of ion sensing.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

This is a review article and no data are available.

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