

Hydrogen-Bonding Interactions between Water and the One- and Two-Electron-Reduced Forms of Vitamin K₁: Applying Quinone Electrochemistry To Determine the Moisture Content of Non-Aqueous Solvents

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Abstract: Vitamin K₁ (VK₁) was shown by voltammetry and coulometry to undergo two chemically reversible one-electron reduction processes in acetonitrile (CH₃CN) containing 0.2 M Bu₄NPF₆ as the supporting electrolyte. The potential separation between the first and second electron-transfer steps diminished sequentially with the addition of water, so that at a H₂O concentration of approximately 7 M (~13% v/v) only one process was detected, corresponding to the reversible transfer of two electrons per molecule. The voltammetric behavior was interpreted on the basis of the degree of hydrogen bonding between the reduced forms of VK₁ with water in the solvent. It was found that the potential separation between the first and second processes was especially sensitive to water in the low molar levels (0.001–0.1 M); therefore, by measuring the peak separation as a function of controlled water concentrations (accurately determined by Karl Fischer coulometric titrations) it was possible to prepare calibration curves of peak separation versus water concentration. The calibration procedure is independent of the type of reference electrode and can be used to determine the water content of CH₃CN between 0.01 and 5 M, by performing a single voltammetric scan in the presence of 1.0 mM VK₁. The voltammetry was also investigated in dichloromethane, dimethylformamide, and dimethyl sulfoxide. The reduction processes were monitored by in situ electrochemical UV–vis spectroscopy in CH₃CN over a range of water concentrations (0.05–10 M) to spectroscopically identify the hydrogen-bonded species.

1. Introduction

Vitamin K comprises a series of lipophilic natural compounds that are produced by plants and bacteria and that are based on 2-methyl-1,4-naphthoquinone derivatives, with an aliphatic side chain in the 3-position.¹ Vitamin K₁ (VK₁; also known as phyloquinone) contains four isoprenoid groups in its side chain, one of which is unsaturated (Scheme 1a). Vitamin K₂ (also called menaquinone) has a side chain with a variable number of unsaturated isoprenoid groups. The important biological properties of vitamin K involve proton and electron transfers and are controlled by the naphthaquinone functional group, while the side chain provides compatibility with the hydrophobic membrane, similar to the function of the phytyl chain in vitamin E and other lipid soluble compounds.²

Vitamin K is an important cofactor involved in blood clotting.¹ The mechanism is believed to involve the reversible formation of the hydroquinone in a two-electron, two-proton process in the presence of a suitable proton donor/acceptor and reducing/oxidizing agent (Scheme 1b), in an analogous electrochemical mechanism that occurs for most quinones/hydro-

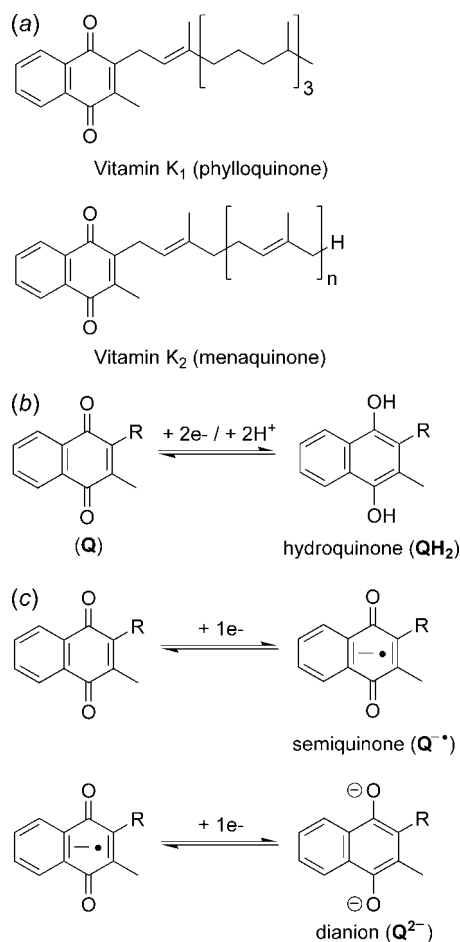
quinones.³ In photosystem I, vitamin K₁ is thought to act as an intermediate one-electron acceptor via the formation of the semiquinone radical anion (Q^{•-}), which aids in electron transfer across a photosynthetic membrane (Scheme 1c).⁴ Because vitamin K and its reduced forms exist inside hydrophobic cell membranes, it is beneficial to study its electrochemical properties in a low water content environment, such as aprotic organic solvents, where the electrochemistry differs from that observed in aqueous systems.³ It is also important to examine the interactions between reduced forms of vitamin K and potential hydrogen-bonding donors (including trace water) which may significantly influence its biological redox properties.

(3) (a) Hammerich, O.; Svensmark, B. In *Organic Electrochemistry*, 3rd ed.; Lund, H., Baizer, M. M., Eds.; Marcel Dekker: New York, 1991; Chapter 16. (b) Chambers, J. Q. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. II, Chapter 12, pp 719–757.

(4) (a) Blankenship, R. E. *Molecular Mechanisms of Photosynthesis*, 1st ed.; Blackwell Science: Oxford, 2002. (b) Trumpower, B. L. *Function of Quinones in Energy Conserving Systems*; Academic Press: New York, 1982. (c) Voet, D.; Voet, J. G. *Biochemistry*, 3rd ed.; Wiley: New York, 2004. (d) Thurnauer, M. C.; Brown, J. W.; Gast, P.; Feezel, L. L. *Radiat. Phys. Chem.* **1989**, *34*, 647–651. (e) Van der Est, A. *Biochim. Biophys. Acta* **2001**, *1507*, 212–225. (f) Rigby, S. E. J.; Evans, M. C. W.; Heathcote, P. *Biochim. Biophys. Acta* **2001**, *1507*, 247–259. (g) Epel, B.; Niklas, J.; Sinnecker, S.; Zimmermann, H.; Lubitz, W. *J. Phys. Chem. B* **2006**, *110*, 11549–11560.

(1) *Vitamin K and Vitamin K-Dependent Proteins: Analytical, Physiological, and Clinical Aspects*; Shearer, M. J., Seghatchian, M. J., Eds.; CRC Press: Boca Raton, FL, 1993.

(2) Wang, X. Y.; Quinn, P. J. *Mol. Membr. Biol.* **2000**, *17*, 143–156.

Scheme 1. Different Forms of Vitamin K and Their Electrochemical Reduction Mechanism

The differences in the electrochemical behavior of quinones in aqueous (buffered and unbuffered) and non-aqueous solvents have recently been rationalized on the basis of proton transfer and hydrogen-bonding mechanisms.^{5a} In buffered aqueous solutions at pH < ~7, quinones (Q) are reduced in a 2e⁻/2H⁺ process to reversibly form the hydroquinones (QH₂) (Scheme 1b).³ The observed potential (E_{obs}) of the process varies according to the pH because protons are involved in the reduction reaction. Hence, the measured E_{obs} is shifted from the formal potential (E_f°) according to the Nernst equation:⁶

$$E_{\text{obs}} = E_f^\circ + \frac{2.303RT}{nF} \times \log \frac{[Q][H^+]^2}{[QH_2]} \quad (1)$$

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 kelvins), and F is the Faraday constant (96485 C mol⁻¹). When [Q] = [QH₂], then $E_{\text{obs}} = E_{1/2}^\circ$ (the reversible half-wave potential). According to eq 1, at 25 °C the $E_{1/2}^\circ$ optimally shifts by -59.2 mV per one unit increase in pH (providing the E_f° does not significantly vary with the change in pH). Although the reaction is written as 2e⁻/2H⁺ in one step (concertedly), it is possible that the electron-transfer and proton-transfer steps occur consecutively or by a mixed concerted/consecutive mechanism.

As the pH increases above ~7, the semiquinone (Q^{•-}) and dianion (Q²⁻) have increased lifetimes, meaning that the acid dissociation constants of their associated protonated forms need to be taken into account in the Nernst equation.^{5a} Thus, the relationship between the E_{obs} of the quinone and the proton concentration can be given by eq 2, where K_{a1} and K_{a2} are the acid dissociation constants of QH₂ and QH⁻, respectively. In nonbuffered aqueous solutions, the localized pH at the electrode surface can change because of the gain or loss of protons from the quinone/hydroquinone system and the interpretation of the voltammetry is complicated.^{3,5,7}

$$E_{\text{obs}} = E_f^\circ + \frac{2.303RT}{nF} \times \log \left(1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}} \right) \quad (2)$$

In aprotic organic solvents containing no added acid, the quinone undergoes one-electron reduction to the semiquinone, which can be further reduced by one electron at more negative potentials to form the dianion (Q²⁻) (Scheme 1c).^{3,5,8-19} The voltammetry has been shown to be heavily influenced by water in the solvent as the potential of both the first reduction process (E_1/V) and especially the second reduction process (E_2/V) shift to more positive potentials with increasing water concentration ($\Delta E_2 > \Delta E_1$). Eventually at high water concentrations the second process merges with the first process, corresponding to the reversible transfer of two electrons per molecule. It is believed that the shift in potential of the two processes is too great to be caused by protonation effects as occur in eqs 1 and 2 (because the pH of the solution does not significantly change). Instead, it has been proposed that the shift in E_1 and E_2 with increasing water is due to an equilibrium that exists between the anionic

- (6) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; Wiley: New York, 2001.
- (7) (a) Müller, O. H. *J. Am. Chem. Soc.* **1940**, *62*, 2434–2441. (b) Kolthoff, I. M.; Orlemann, E. F. *J. Am. Chem. Soc.* **1941**, *63*, 664–667. (c) Abbott, J. C.; Collat, J. W. *Anal. Chem.* **1963**, *35*, 859–863. (d) Bailey, S. I.; Ritchie, I. M. *Electrochim. Acta* **1985**, *30*, 3–12. (e) Robertson, R. T.; Pendley, B. D. *J. Electroanal. Chem.* **1994**, *374*, 173–177. (f) Sato, Y.; Fujita, M.; Mizutani, F.; Uosaki, K. *J. Electroanal. Chem.* **1996**, *409*, 145–154. (g) Shim, Y.-B.; Park, S.-M. *J. Electroanal. Chem.* **1997**, *425*, 201–207. (h) Park, H.; Won, M.-S.; Cheong, C.; Shim, Y.-B. *Electroanalysis* **2002**, *14*, 1501–1507. (i) Tang, Y.; Wu, Y.; Wang, Z. *J. Electrochem. Soc.* **2001**, *148*, E133–E138. (j) Forster, R. J.; O’Kelly, J. P. *J. Electroanal. Chem.* **2001**, *498*, 127–135.
- (8) (a) Peover, M. E. *J. Chem. Soc.* **1962**, 4540–4549. (b) Peover, M. E.; Davies, J. D. *J. Electroanal. Chem.* **1963**, *6*, 46–53. (c) Peover, M. E.; Davies, J. D. *Trans. Faraday Soc.* **1964**, *60*, 476–478. (d) Peover, M. E. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1967; pp 1–51.
- (9) Umemoto, K. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1058–1065.
- (10) Eggins, B. R. *Chem. Commun.* **1969**, 1267–1268.
- (11) Hayano, S.; Fujihira, M. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 2051–2055.
- (12) Nagaoka, T.; Okazaki, S.; Fujinaga, T. *J. Electroanal. Chem.* **1982**, *133*, 89–99.
- (13) Ashnagar, A.; Bruce, J. M.; Dutton, P. L.; Prince, R. C. *Biochim. Biophys. Acta* **1984**, *801*, 351–359.
- (14) Wilford, J. H.; Archer, M. D. *J. Electroanal. Chem.* **1985**, *190*, 271–277.
- (15) Rüssel, C.; Jaenicke, W. *J. Electroanal. Chem.* **1986**, *199*, 139–151.
- (16) Laviron, E. *J. Electroanal. Chem.* **1986**, *208*, 357–372.
- (17) Gupta, N.; Linschitz, H. *J. Am. Chem. Soc.* **1997**, *119*, 6384–6391.
- (18) (a) González, F. J. *Electroanalysis* **1998**, *10*, 638–642. (b) Gómez, M.; González, F. J.; González, I. *Electroanalysis* **2003**, *15*, 635–645. (c) Gómez, M.; González, F. J.; González, I. *J. Electrochem. Soc.* **2003**, *150*, E527–E534. (d) Gómez, M.; Gómez-Castro, C. Z.; Padilla-Martínez, I. I.; González, F. J. *J. Electroanal. Chem.* **2004**, *567*, 269–276. (e) Macías-Ruvalcaba, N. A.; González, I.; Aguilar-Martínez, M. *J. Electrochem. Soc.* **2004**, *151*, E110–E118. (f) Gómez, M.; González, F. J.; González, I. *J. Electroanal. Chem.* **2005**, *578*, 193–202.
- (19) (a) Lehmann, M. W.; Evans, D. H. *J. Electroanal. Chem.* **2001**, *500*, 12–20. (b) Lehmann, M. W.; Evans, D. H. *J. Phys. Chem. B* **2001**, *105*, 8877–8884.

(5) (a) Quan, M.; Sanchez, D.; Wasyliw, M. F.; Smith, D. K. *J. Am. Chem. Soc.* **2007**, *129*, 12847–12856. (b) Ge, Y.; Miller, L.; Ouimet, T.; Smith, D. K. *J. Org. Chem.* **2000**, *65*, 8831–8838. (c) Ge, Y.; Lilienth, R. R.; Smith, D. K. *J. Am. Chem. Soc.* **1996**, *118*, 3976–3977.

quinones (Q^{•-} and Q^{2•-}) and their hydrogen-bonded forms.^{3,5,8-19} UV-vis and EPR spectroscopic experiments support the hydrogen-bonding mechanism during the reduction of quinones.²⁰

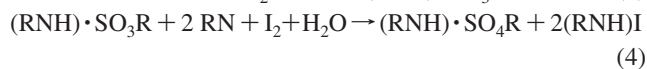
Although hydrogen bonding is known to occur between water and quinone anions and dianions in non-aqueous solvents,^{3,5,8-20} we are not aware of any studies that accurately report the residual water content of the organic solvents. While it is commonly known that the water content of a “dry” solvent is not negligible, it is often assumed that the voltammetric responses are unaffected by very low levels of water. This is because it could be expected that the solvent–substrate interactions would be more significant than trace water–substrate interactions, which is true in situations where the non-aqueous solvents undergo strong hydrogen bonding with the substrate. However, it will be demonstrated in this study that differences of a few millimolar H₂O in the solvents CH₃CN, DMF, and DMSO are surprisingly sufficient to change the appearance and potential of the reduction processes of VK₁, and the differences are most pronounced at the lowest water concentrations (0–0.01 M).

Unless scrupulous care is taken in preparing the cells for electrochemical experiments, it is likely that the water content of the solvent will be much greater than that of the substrate,²¹ especially in high humidity environments.²² Solvents that are taken from distillation apparatus or from above molecular sieves will rapidly begin adsorbing water from the atmosphere as soon as they are added to the cells. This is due to limitations in the design of electrochemical cells that require numerous ports for electrodes and purging devices and require the working electrode to be easily removed for polishing.²³ Aqueous reference electrodes will immediately add moisture to the analyte solution, and even non-aqueous reference electrodes are a significant source of water. The supporting electrolytes used for non-aqueous electrochemistry are often hygroscopic and should be dried by heating under vacuum immediately before use.²³

In the present study, the water concentration in the solvent in the electrochemical cell was accurately measured by performing Karl Fischer (KF) titrations. Voltammograms of vitamin K₁ were recorded, and the water content was simultaneously adjusted over relatively small concentration changes (~0.005 M). It was determined that, at water concentrations <0.1 M, the peak separation between E₁ and E₂ was sensitive to a 0.001 M change in (water) concentration. The electrochemical results were complemented with results from in situ UV-vis spectroscopy over a range of water concentrations to identify the hydrogen-bonded anions.

The KF titration is the standard method for obtaining the water content of various substances.²⁴ It is based on the solution-phase reactions given in eqs 3 and 4 and can be performed in coulometric or volumetric mode. ROH in eq 3 is an alcohol,

usually methanol or ethanol, and RN in eq 3 is an amine such as imidazole.



Equation 4 shows that there is a 1:1 reaction between I₂ and H₂O; thus, if the amount of I₂ is accurately known, then the amount of water can be determined. In KF coulometric titrations (as performed in this study), the I₂ is generated electrochemically by the oxidation of iodide (eq 5).



According to eqs 4 and 5, 2 mol of electrons is produced for every mole of water that reacts with iodine. Therefore, 2 × 96485 coulombs (C) are produced for 1 mol of water present, or stated another way: 1 μg of water corresponds to 0.01072 C passed. The end point of the titration is measured by a change in voltage across two indicator electrodes due to the presence of free iodine.

The great advantage of the KF titration is that it does not need calibration, since the charge can be directly correlated with the amount of water present. However, some limitations exist; in particular, there is a minimum charge that can be accurately measured by the coulometer, meaning that low concentrations of water require higher masses of the analyte to achieve the greatest accuracy. With care the KF titration can accurately give measurements down to 1 ppm water. In this study, most measurements were conducted between 200 and 2000 ppm water, a range over which reliable readings can be easily obtained. In certain situations, the charge measured during the oxidation of iodine is not necessarily selective for water because other oxidizable species are present, or the reaction in eq 4 between I₂ and H₂O does not occur in a 1:1 ratio.²⁴ Nevertheless, the solvents/electrolytes used in this study represent straightforward medium within which to calculate the water content without any known special difficulties.

2. Experimental Section

2.1. Chemicals. Vitamin K₁ (98%) (Sigma-Aldrich) was used as received. Bu₄NPF₆ was prepared by a standard method,²³ dried under vacuum at 413 K for 24 h, and stored under vacuum. HPLC grade CH₃CN (Tedia) and CH₂Cl₂ (Fischer Scientific) were initially dried by distilling over CaH₂ (under nitrogen) immediately before use, while DMSO (Tedia) and DMF (Tedia) were used from the bottles.

2.2. Measurement of Water Content of Organic Solvents. KF titrations were conducted with a Mettler Toledo DL32 coulometer using (Riedel-deHaën) HYDRANAL-coulomat CG for the cathode compartment and HYDRANAL-coulomat AG for the anode compartment. The instrument was first allowed to stabilize until the drift was 0 μg min⁻¹ H₂O, which usually required 30 min of operation. The samples were injected into the coulometer using plastic disposable syringes through a silicon/Teflon septum. Each measurement took less than 1 min, meaning that the drift from atmospheric water entering the coulometer was negligible.

2.3. Voltammetry. Cyclic voltammetry (CV) and square-wave voltammetry (SWV) experiments were conducted with a computer-controlled Eco Chemie μAutolab III potentiostat. Working electrodes were 1-mm diameter planar Pt and glassy carbon (GC) disks (Cypress Systems), used in conjunction with a Pt auxiliary electrode (Metrohm) and a Ag wire miniature reference electrode (Cypress Systems) connected to the test solution via a salt bridge containing 0.5 M Bu₄NPF₆ in CH₃CN. Accurate potentials were obtained using ferrocene as an internal standard.

- (20) (a) Stone, E. W.; Maki, A. H. *J. Chem. Phys.* **1962**, *36*, 1944–1945. (b) Gendell, J.; Freed, J. H.; Fraenkel, G. K. *J. Chem. Phys.* **1962**, *37*, 2832–2841. (c) Fujihira, M.; Hayano, S. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 644–645.
- (21) (a) Burfield, D. R.; Lee, K. H.; Smithers, R. H. *J. Org. Chem.* **1977**, *42*, 3060–3065. (b) Burfield, D. R.; Gan, G.-H.; Smithers, R. H. *J. Appl. Chem. Biotechnol.* **1978**, *28*, 23–30.
- (22) The mean relative humidity in Singapore is 84%, typically ranging from >90% in the early morning to 60% in the late afternoon.
- (23) Fry, A. J.; Britton, W. E. In *Laboratory Techniques in Electroanalytical Chemistry*; Kissinger, P. T., Heineman, W. R., Eds.; Marcel Dekker: New York, 1984; Chapter 13.
- (24) Scholz, E. In *Karl Fischer Titration: Determination of Water*; Springer-Verlag: Berlin, 1984.

2.4. Method for Drying Solutions for Electrochemistry. All solutions for electrochemistry were dried by placing the solvent (CH_3CN and CH_2Cl_2 were first predried over CaH_2), electrolyte, and vitamin K_1 inside a 25-mL vacuum syringe (SGE Analytical Science) containing 3 Å molecular sieves (that were dried under vacuum at 513 K for 12 h) and storing the syringe under a nitrogen atmosphere for at least 48 h. The VK_1 was stable under these conditions in each of the solvents tested, even after several days directly in contact with the molecular sieves, since the peak currents measured by voltammetry were the same before and after drying. The syringe was wrapped in aluminum foil during the drying process to prevent photochemical reactions of VK_1 .

It was found (by KF titration) that after 48 h the water content of the solvent/electrolyte/vitamin inside the syringe was between 0.0005 and 0.0015 M (~ 10 –35 ppm). The contents of the syringe were then injected through a 0.2 μM disposable filter (Sartorius Stedim Biotech, Minisart) (that had been dried under vacuum at 50 °C for 2 h) into a glass electrochemical cell that had been dried at 100 °C in an oven and then allowed to cool under an argon atmosphere. KF titrations indicated that the water content increased to 0.007 ± 0.003 M as a result of the transfer from the syringe to the electrochemical cell. After deoxygenating the solution for 10 min with argon gas, the voltammetric scans were commenced, with an aliquot of solvent being simultaneously tested by KF titration. A Pt wire was used as the pseudo-reference electrode for experiments with the lowest water concentrations, which was replaced with the Ag wire miniature reference electrode when the water content was increased above 0.05 M. At concentrations of water >0.1 M, the water content was calculated on the basis of the accurately known volume of added water.

2.5. Controlled Potential Electrolysis. Experiments were performed in a divided controlled potential electrolysis cell separated with a porosity no. 5 (1.0–1.7 μm) sintered glass frit.²⁵ The working and auxiliary electrodes were identically sized Pt mesh plates symmetrically arranged with respect to each other with a Ag wire reference electrode (isolated by a salt bridge) positioned to within 2 mm of the surface of the working electrode. The volumes of both the working and auxiliary electrode compartments were approximately 10 mL each. The solution in the working electrode compartment was simultaneously deoxygenated and stirred using bubbles of argon gas. The number of electrons transferred during the bulk oxidation process was calculated from

$$N = Q/nF \quad (6)$$

where N = no. of moles of starting compound and Q = charge (coulombs).

2.6. In Situ UV–Vis Spectroscopy. A Perkin-Elmer Lambda 750 spectrophotometer was used in conjunction with an optically semitransparent thin layer electrochemical (OSTLE) cell (path length = 0.05 cm) using a Pt mesh working electrode.²⁶ Variable-temperature (253–293 K) experiments were controlled with a Thermo Electron Neslab RTE 740 circulating bath containing propan-2-ol. The temperature of the OSTLE cell was controlled using a PerkinElmer flow cell connected to the circulating bath. The temperature difference between the circulating bath and the cell was calibrated using a thermocouple inside the OSTLE cell. The cavity of the spectrometer was purged from the atmosphere with a high volume flow of nitrogen gas.

2.7. Theoretical Calculations. Digital simulations of the CV data were performed using the DigiElch software package.²⁷

3. Results and Discussion

3.1. Electrochemistry at Intermediate (0.05 M) to High (10 M) Water Concentrations. Figure 1 shows CVs of VK_1 in CH_3CN obtained at GC and Pt electrode surfaces at variable

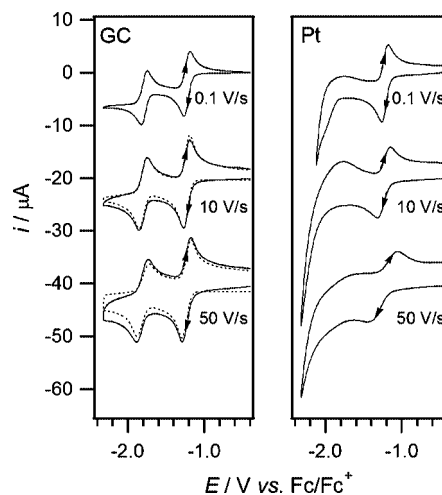


Figure 1. Variable scan rate CVs recorded at 293 K at 1-mm diameter planar GC and Pt working electrodes in CH_3CN containing 1 mM VK_1 , 0.2 M Bu_4NPF_6 , and 0.05 (± 0.01) M H_2O . The current data were normalized by multiplying by $\nu^{-0.5}$ (ν = scan rate). Data at 10 and 50 V s^{-1} are offset by -20 and -40 μA , respectively. (···) Simulations recorded with the following parameters (eqs 7–10): $E_1 = -1.23$ V ($k_{s(1)} = 1$ cm s^{-1}), $E_2 = -1.80$ V ($k_{s(2)} = 0.1$ cm s^{-1}), $D = 2.0 \times 10^{-5}$ $\text{cm}^2 \text{s}^{-1}$, $R = 320$ Ω , $C_d = 1 \times 10^{-7}$ F cm^{-2} , $k_{f(1)} = 1 \times 10^7$ L $\text{mol}^{-1} \text{s}^{-1}$, $k_{b(1)} = 1 \times 10^3$ s^{-1} , $K_{\text{eq}(1)} = 1 \times 10^5$, $k_{f(2)} = 1 \times 10^{-3}$ s^{-1} , $k_{b(2)} = 1.6 \times 10^{-9}$ L $\text{mol}^{-1} \text{s}^{-1}$, $K_{\text{eq}(2)} = 6.4 \times 10^5$.

scan rates in the presence of 0.05 (± 0.01) M H_2O . On GC, two one-electron reduction processes were detected corresponding to the chemically reversible transformation of VK_1 to the monoanion ($\text{VK}_1^{\cdot-}$) (eq 7) and dianion (VK_1^{2-}) (eq 8).



$E_{f(1)}^\circ$ and $E_{f(2)}^\circ$ refer to the formal electrode potentials, which for quinones are strongly dependent on the water content of the solvent; thus, absolute values can only be reported if the water content is accurately known. The E_f° values can be approximated from the $E_{1/2}^{\text{r}}$ values obtained during CV experiments, which for electrochemically reversible processes can be measured from the midpoint of the cathodic (E_p^{red}) to anodic (E_p^{ox}) peak potentials in situations where the cathodic (i_p^{red}) and anodic (i_p^{ox}) peak current ratios are equal to unity.⁶

On GC surfaces at a scan rate (ν) of 100 mV s^{-1} , the anodic to cathodic peak-to-peak separation (ΔE_{pp}) for both processes was 75 mV, similar to the value obtained for ferrocene under identical conditions. However, as the scan rate was increased, the ΔE_{pp} values for the two processes diverged, so that at $\nu = 50$ V s^{-1} , the ΔE_{pp} values for the first and second processes were 115 and 150 mV, respectively. The increase in ΔE_{pp} value for the first process at faster scan rates can be accounted for by the effects of uncompensated solution resistance and was similar

(26) (a) Webster, R. D.; Heath, G. A.; Bond, A. M. *J. Chem. Soc., Dalton Trans.* **2001**, 3189–3195. (b) Webster, R. D.; Heath, G. A. *Phys. Chem. Chem. Phys.* **2001**, 3, 2588–2594.

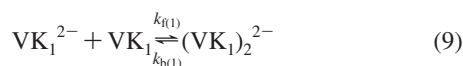
(27) (a) Rudolph, M. *J. Electroanal. Chem.* **2003**, 543, 23–39. (b) Rudolph, M. *J. Electroanal. Chem.* **2004**, 571, 289–307. (c) Rudolph, M. *J. Electroanal. Chem.* **2003**, 558, 171–176. (d) Rudolph, M. *J. Comput. Chem.* **2005**, 26, 619–632. (e) Rudolph, M. *J. Comput. Chem.* **2005**, 26, 633–641. (f) Rudolph, M. *J. Comput. Chem.* **2005**, 26, 1193–1204.

(25) Webster, R. D.; Bond, A. M.; Schmidt, T. *J. Chem. Soc., Perkin Trans.* **2** **1995**, 1365–1374.

to that observed for ferrocene at an identical scan rate (which is known to undergo fast heterogeneous electron transfer²⁸).

The large ΔE_{pp} value observed for the second process at $\nu = 50 \text{ V s}^{-1}$ is greater than can be accounted for by the effects of solution resistance (which was not experimentally compensated for, but was incorporated into the digital simulations) and is instead likely to be caused by a relatively slow rate of electron transfer. The effects of slow heterogeneous electron transfer are even more pronounced on Pt surfaces where the second process is difficult to detect even at slow scan rates, while the ΔE_{pp} value for the first process at $\nu = 50 \text{ V s}^{-1}$ was much greater than that observed on GC at the same scan rate. The second process is also difficult to detect on Pt surfaces at intermediate to high water concentrations because of the kinetic ease of reduction of water on Pt electrodes (hence the large background current shown in Figure 1 on Pt at potentials more negative than -2 V). Because simpler voltammetric behavior was obtained on GC compared to Pt surfaces, the remaining discussion in this article is restricted to the GC electrode.

The CVs on GC in Figure 1 illustrate that the current observed for the second process becomes smaller in relation to the first process as the scan rate is increased. It was previously observed in quinone electrochemistry that the height of the second peak is often less than the first, and it was explained on the basis of a complexation reaction between the dianion and the starting material (eq 9).¹⁷



For eq 9 to be observable voltammetrically, it requires that the thermodynamically favorable monomerization reaction to form the anion radicals (eq 10, $k_{f(2)}$) to be relatively slow compared to the complexation reaction (eq 9, $k_{f(1)}$). Furthermore, the dimerization reaction of the anion radicals (eq 10 $k_{b(2)}$) (which is a mechanism known to occur for the anion radicals of 9-substituted anthracenes²⁹ and aromatic esters³⁰) must be slower than the monomerization reaction. Digital simulations of the voltammetric data in Figure 1 based on eqs 7–10 allowed estimations of the rate constants for eqs 9 and 10, which are provided in the Figure 1 caption. The simulations indicate that the complexation reaction (eq 9) is feasible, although the kinetic values are only approximate because the observed $E_{1/2}^r$ values (and hence the kinetic data) are heavily influenced by the exact solvent composition.

Controlled potential electrolysis experiments were conducted on VK₁ to determine the lifetimes of the reduced states and to confirm the number of electrons transferred during the reduction

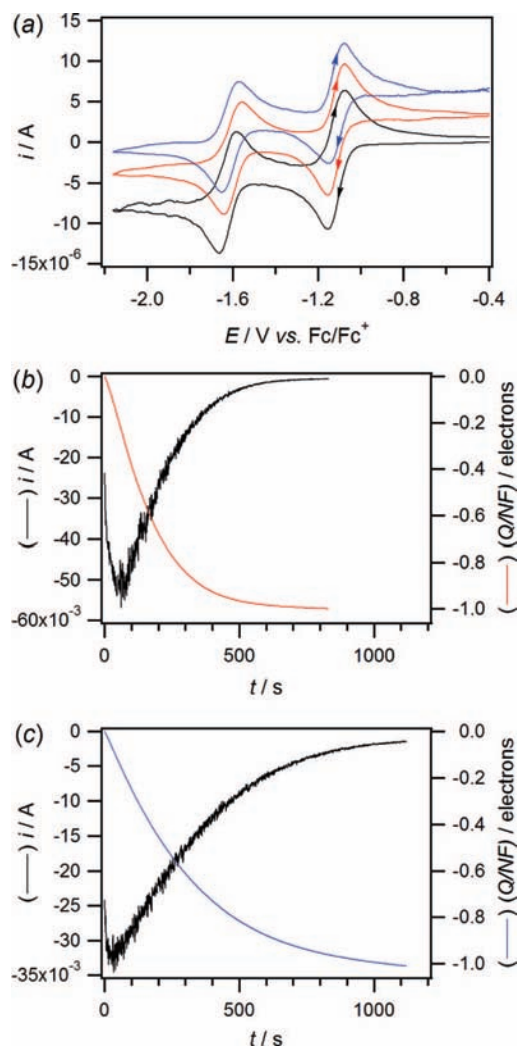


Figure 2. Voltammetric and coulometric data obtained at 293 K during the controlled potential electrolysis of 5 mM VK₁ in CH₃CN with 0.2 M Bu₄NPF₆ and 0.10 (± 0.01) M H₂O. (a) CVs recorded at a scan rate of 0.1 V s⁻¹ with a 1-mm diameter GC electrode. (Black line) Before the bulk reduction of VK₁. (Red line) After the exhaustive reduction of VK₁ at an applied potential of $-1.4 \text{ V vs Fc/Fc}^+$. (Blue line) After the reduction of VK₁^{•-} at an applied potential of $-1.8 \text{ V vs Fc/Fc}^+$. (b) Current/coulometry vs time data obtained during the exhaustive reduction of VK₁ at $-1.4 \text{ V vs Fc/Fc}^+$. (c) Current/coulometry vs time data obtained during the exhaustive reduction of VK₁^{•-} at $-1.8 \text{ V vs Fc/Fc}^+$.

processes over longer times. Figure 2 shows CVs and coulometry data obtained at various intervals during the reduction of VK₁ in CH₃CN containing 0.10 (± 0.01) M water. Under electrolysis conditions, it was very difficult to further reduce the amount of water present because of the requirement of multiple compartments in the electrolysis cell.

Figure 2a shows CVs obtained before (black line) and after the one-electron (red line) and two-electron (blue line) reduction of VK₁. For both processes (E_1 and E_2), the total current measured between the i_p^{red} and i_p^{ox} values remained constant, indicating that the reduced forms were stable on the timescale of the electrolysis experiments. The current values observed during CV experiments were offset in the positive current direction after the electrolysis experiments were commenced because of the change in oxidation state of the VK₁ species present in the bulk solution. Figure 2b,c shows the corresponding current and coulometry data obtained during the electrolysis of

(28) Bond, A. M.; Henderson, T. L. E.; Mann, D. R.; Mann, T. F.; Thormann, W.; Zoski, C. G. *Anal. Chem.* **1988**, *60*, 1878–1882.

(29) (a) Hammerich, O.; Parker, V. D. *Acta Chem. Scand., Ser. B* **1981**, *35*, 341–347. (b) Parker, V. D. *Acta Chem. Scand., Ser. B* **1981**, *35*, 349–356. (c) Hammerich, O.; Parker, V. D. *Acta Chem. Scand., Ser. B* **1983**, *37*, 379–392. (d) Hammerich, O.; Parker, V. D. *Acta Chem. Scand., Ser. B* **1983**, *37*, 851–856. (e) Amatore, C.; Pinson, J.; Savéant, J. M. *J. Electroanal. Chem.* **1982**, *137*, 143–148. (f) Amatore, C.; Pinson, J.; Savéant, J. M. *J. Electroanal. Chem.* **1982**, *139*, 193–197. (g) Amatore, C.; Garreau, D.; Hammi, M.; Pinson, J.; Savéant, J. M. *J. Electroanal. Chem.* **1985**, *184*, 1–24. (h) Smith, C. Z.; Utey, J. H. P. *J. Chem. Res., Synop.* **1982**, 18–19. (i) Mendkovich, A. S.; Michalchenko, L. V.; Gulytai, V. P. *J. Electroanal. Chem.* **1987**, *224*, 273–275.

(30) Webster, R. D. *J. Chem. Soc., Perkin Trans. 2* **1999**, 263–269.

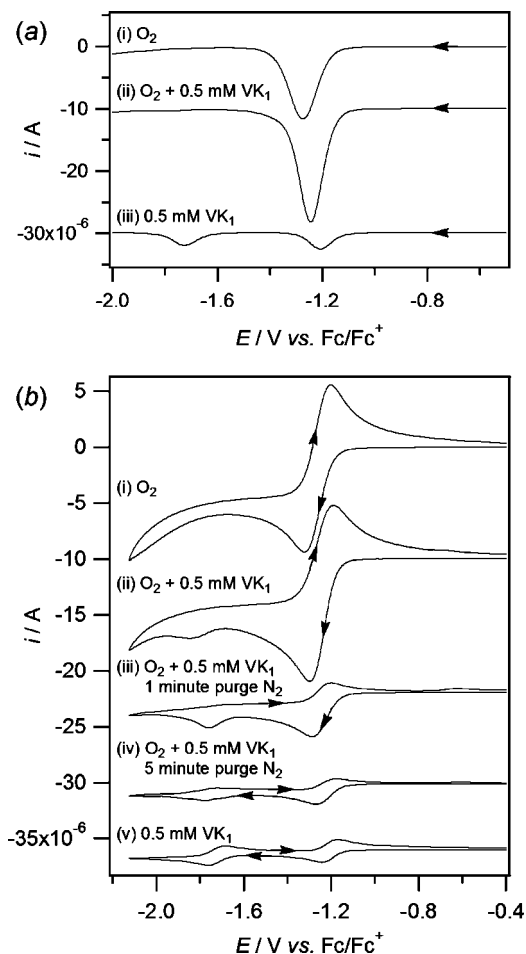


Figure 3. Voltammograms recorded at a 1-mm diameter planar GC electrode in CH₃CN containing 0.2 M Bu₄NPF₆ and 0.05 (±0.01) M H₂O at 293 K showing the effect of dissolved molecular oxygen on the voltammetry of 0.5 mM VK₁. (a) SWVs recorded with a pulse period (τ) = 25 Hz, a potential step = 2 mV, and a pulse amplitude = 20 mV. (b) CVs recorded at a scan rate of 100 mV s⁻¹.

VK₁ and VK₁^{•-}, respectively, confirming that one electron per molecule was transferred in each electrochemical step.

It was found that the electrochemistry of VK₁ was affected by low levels of dissolved molecular oxygen. Oxygen can be electrochemically reduced by one electron to superoxide at -1.25 V (±0.2) vs Fc/Fc⁺ in CH₃CN, with a half-life of around 1 h at 20 °C.³¹ Figure 3a(i),b(i) shows a SWV and CV, respectively, of the reduction of dissolved molecular oxygen present in atmospheric concentrations (2–3 mM). When 0.5 mM VK₁ was added to solutions containing atmospheric concentrations of O₂, SWV experiments indicated that the first cathodic peak shifted to more positive potentials by ~+30 mV and the peak current increased (Figure 3a(ii)). Thus, the SWV results indicate that the first reduction process (E_1) for VK₁ in CH₃CN (containing relatively low levels of H₂O) is sufficiently close to the formal reduction potential of O₂ to render their reduction processes indistinguishable from each other in CH₃CN containing both species (VK₁ and O₂).

It was also observed that the second reduction process of VK₁ (E_2) appeared chemically irreversible when CVs were performed in solutions of VK₁ in the presence of O₂ (Figure 3b(iii),(iv)).

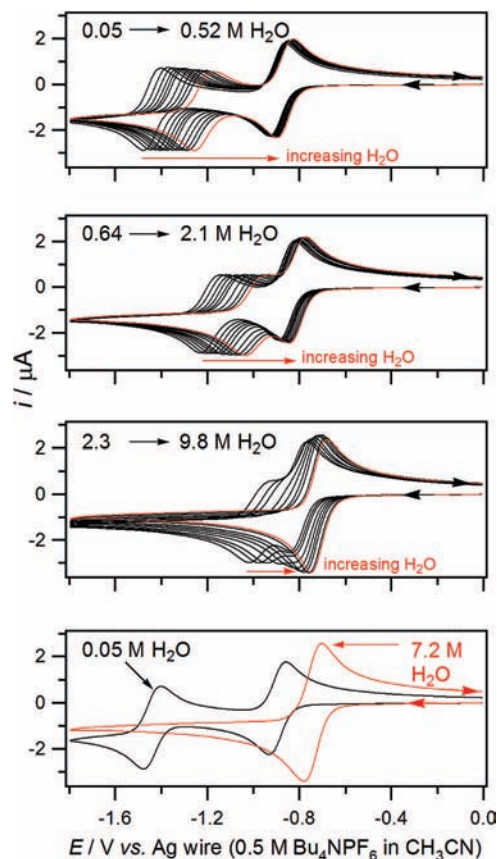


Figure 4. CVs recorded of VK₁ (initial concentration = 1.0 mM, final concentration = 0.83 mM) at a scan rate of 0.1 V s⁻¹ at a 1-mm diameter planar GC electrode in CH₃CN containing 0.2 M Bu₄NPF₆ at 293 K, with varying concentrations of water.

It was only when the solution had been extensively deoxygenated that the first and second reduction processes both appeared fully chemically reversible during CV experiments (Figure 3b(v)). The amount of time needed for deoxygenating depended on the concentration of VK₁, the rate of purging of the argon gas, and the solution volume. The higher the concentration of VK₁ the less purging time was required because the amount of dissolved oxygen was relatively lower compared to the VK₁ concentration. Under our conditions, it was found that 10 min of purging was required to remove molecular oxygen.

At very low concentrations of VK₁ (<0.1 mM), it was found that the second process appeared much smaller than the first process (ratio <0.2: 1), which is possibly caused by residual O₂ (or O₂^{•-}) reacting with VK₁^{•-} (or VK₁), which could not be satisfactorily removed with purging. Furthermore, during SWV experiments at VK₁ concentrations > 0.5 mM, the second reduction process always appeared smaller than the first (ratio of 0.8:1), which is likely to be caused by the slower heterogeneous electron-transfer rate for the second process ($k_{s(2)} \approx 0.1$ cm s⁻¹) and the complexation reaction of the dianion with the quinone (eq 9) affecting the peak height of the SWV.

Figure 4 shows CVs obtained for VK₁ in carefully deoxygenated solutions of CH₃CN in the presence of varying H₂O concentrations. It can be observed that both the first and second processes shift to more positive potentials with increasing water concentrations, but the second process shifts by a greater amount ($\Delta E_2 > \Delta E_1$) so that the processes eventually merge into one at a H₂O concentration of approximately 7.2 M (corresponding

(31) Webster, R. D.; Bond, A. M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1075–1079.

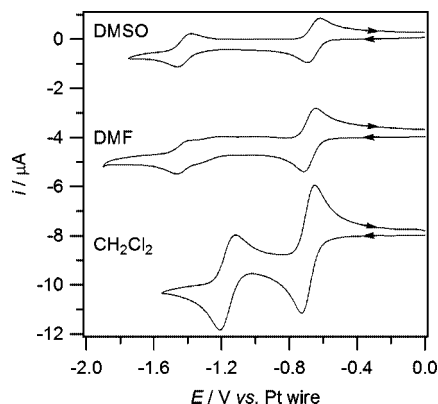


Figure 5. CVs recorded at 293 K at a 1-mm diameter planar GC working electrode of solutions containing 1 mM VK₁, 0.2 M Bu₄NPF₆, and 0.05 (± 0.01) M H₂O. Data in DMF and CH₂Cl₂ are offset by -4 and -8 μ A, respectively.

to a two-electron reduction where the two one-electron transfers occur in rapid succession at similar potentials).

On the basis of the CV results, it can be concluded that both the monoanion and dianion undergo hydrogen bonding with water in the solvent, but the very large shift observed for the second process would suggest that the dianion undergoes much stronger hydrogen bonding. It would be expected that the strongly electronegative oxygen atoms in the dianion are more likely to interact with water molecules than the less electronegative oxygen atoms in the monoanion. Theoretical calculations³² on quinone anion radicals and dianions have indicated that the oxygen atoms do have an increasing negative charge density in moving from the neutral molecule to the semiquinone and then dianion.^{32c} Because of the relatively large shift in potential of E_2 with small additions of water, it is questionable whether the dianion actually exists in “non-aqueous” solvents in a non-hydrogen-bonded form, since the water content of the solvent is usually much greater than that of the analyte.²³

Figure 5 shows CV data obtained for 1 mM VK₁ in DMSO, DMF, and CH₂Cl₂, which show some similarities and differences with the CVs obtained in CH₃CN. The $|E_1 - E_2|$ separations observed in DMSO and DMF containing 0.05 M H₂O were greater than those observed in CH₂Cl₂ and CH₃CN by ~ 200 mV. This observation implies that there is less hydrogen bonding between the reduced forms of VK₁ and water in DMSO and DMF compared to that in CH₃CN and CH₂Cl₂. The reason for the difference could relate to strong interactions between water and the solvent reducing the interactions between water and the VK₁ anions. It was reported that the anion radicals of anthracene and naphthalene were longer-lived in DMF and DMSO compared to that in CH₃CN, and it was postulated that strong interactions between the water and DMF/DMSO lowered the protonation rate of the anion radicals with water.^{33a} An NMR study indicated that water–DMSO solutions undergo strong intermolecular interactions that contribute to a structuring of the binary liquid.^{33b}

The peak currents observed in CH₂Cl₂ were greater than those observed in CH₃CN by $\sim 30\%$, while the cathodic peak currents

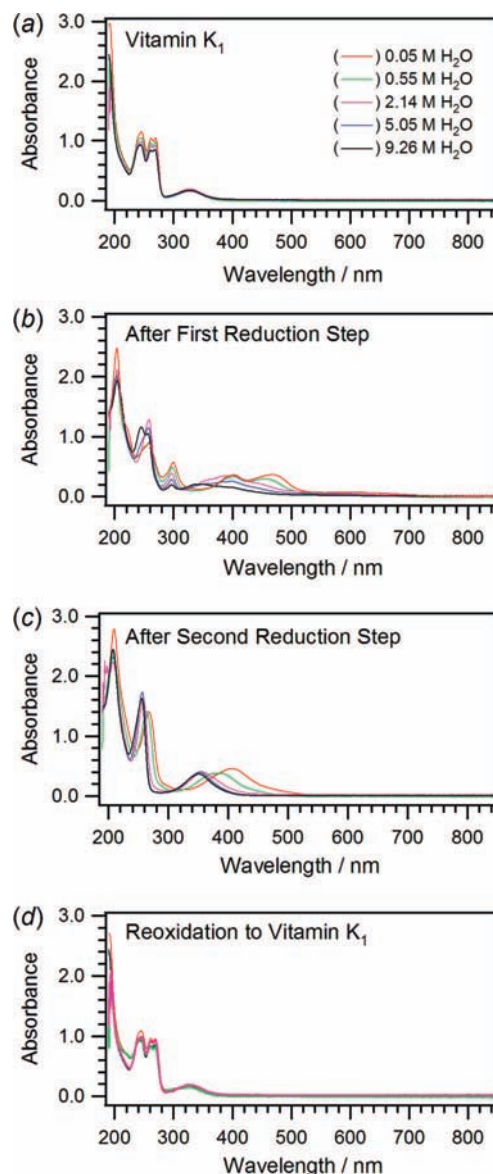


Figure 6. In situ UV–vis spectra obtained in an OSTLE cell during the reduction (and reoxidation) of 1 mM VK₁ at 293 K in CH₃CN with 0.2 M Bu₄NPF₆ and varying concentrations of water.

(i_p^{red}) observed in DMF and DMSO were less than those observed in CH₃CN by factors of 2–3. The differences in the i_p^{red} values are partly due to changes in the diffusion coefficient of VK₁ in the different solvents due to changing solvent viscosities. The size of the solvated molecules will also vary between the different solvents, which will also influence the diffusion coefficient values. For DMF, the second process appeared less chemically reversible than for the other solvents and additional processes were detected before and after the main electron transfer process (around E_2) (Figure 5).

3.2. In Situ UV–Vis Spectroscopy at Intermediate (0.05 M) to High (10 M) Water Concentrations. To further investigate the degree of hydrogen bonding in the monoanion radical and dianion, in situ electrochemical UV–vis spectroscopy experiments were conducted on VK₁ in CH₃CN in the presence of varying concentrations of water (0.05, 0.55, 2.14, 5.05, and 9.26 M H₂O). Figure 6 shows the spectra obtained (a) before reduction, after the (b) first and (c) second reduction steps, and (d) after the reduced compounds had been oxidized back to the

- (32) (a) Mohandas, P.; Umaphathy, S. *J. Phys. Chem. A* **1997**, *101*, 4449–4459. (b) Zhan, C. G.; Chipman, D. M. *J. Phys. Chem. A* **1998**, *102*, 1230–1235. (c) Wheeler, D. E.; Rodriguez, J. H.; McCusker, J. K. *J. Phys. Chem. A* **1999**, *103*, 4101–4112. (d) Tripathi, G. N. R.; Chipman, D. M. *J. Phys. Chem. A* **2002**, *106*, 8908–8916.
- (33) (a) Jezorek, J. R.; Mark, H. B., Jr. *J. Phys. Chem.* **1970**, *74*, 1627–1633. (b) Tokuhiko, T.; Menafra, L.; Szmant, H. H. *J. Chem. Phys.* **1974**, *61*, 2275–2282.

starting material. Irrespective of the concentration of water, it was found that the UV–vis spectrum of the starting material could be obtained after the reoxidation of the anion/dianion, indicating that the reduced forms were stable on the timescale of the experiments (~30 min).

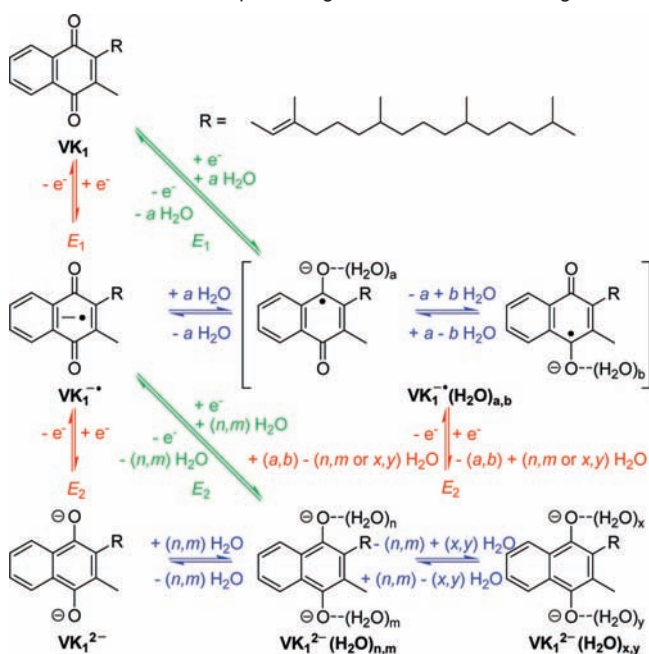
The cyclic voltammograms shown in Figure 4 illustrate that there is a relatively small change in E_1 for water concentrations between 0.05 and 0.5 M. Therefore, it would be expected that, with a H_2O concentration of 0.05 M, the anion radical would exist in a predominantly non-hydrogen-bonded form. Thus, the spectrum of the one-electron-reduced form of VK_1 with 0.05 M water is likely to be the non-hydrogen-bonded semiquinone radical (Figure 6b, red line). As the concentration of water was increased to 0.55 M, a relatively small change in the UV–vis spectrum of the singly reduced species was observed (Figure 6b, green line). As the concentration of water was increased >0.55 M, the spectra began to change substantially, because of increased hydrogen bonding of the semiquinone. It was observed that the band at 485 nm decreased in intensity faster than the band at 400 nm as the water concentration increased, while the sharp band at 300 nm (that appeared characteristic of the non-hydrogen-bonded semiquinone) decreased in intensity with increasing water concentration. The UV–vis results in Figure 6b are consistent with an equilibrium between the hydrogen-bonded and non-hydrogen-bonded semiquinone.

The UV–vis spectra obtained after the second one-electron reduction step (two electrons overall) are given in Figure 6c. Three bands were detected which all moved to lower wavelength with increasing concentration of water, with the greatest shift observed for the band at the highest wavelength. The position of the bands remained close to constant as the water content increased above 5 M H_2O . On the basis of the large shifts in potential observed with increasing water concentrations (Figure 4), it is likely that under the present experimental conditions the dianion *only* exists in the bulk solution in a hydrogen-bonded form (unlike the situation observed for the semiquinone radical). The UV–vis spectra did not display any substantial differences in appearance when the temperature was varied between 293 and 253 K, indicating that any equilibria between the hydrogen-bonded species responsible for the absorbances in Figure 6 were not strongly sensitive to changes in temperature.

It is interesting that, even at very high water concentrations (9.26 M), when only one voltammetric wave was observed, small amounts of the non-hydrogen-bonded semiquinone can still be detected in the UV–vis spectrum, via the band at 300 nm. Because only one voltammetric process was detected in the presence of 9.26 M water, the UV–vis spectra of the species responsible for the first and second electron-transfer steps could not be controlled by adjusting the applied potential. Instead, the spectra were assigned on the basis of the observation that the band at 300 nm initially increased in intensity during the first electron transfer and then diminished in intensity during the second electron transfer. Thus, the spectrum in Figure 6b obtained in the presence of 9.26 M water (black line) was assigned by observing the intensity of the absorbance at 300 nm during the in situ electrolysis.

On the basis of the electrochemical and spectroscopic results in Figures 4 and 6, a mechanism can be proposed for the fates of the reduced species in CH_3CN containing varying concentrations of water, which is essentially a “square scheme” mechanism³ where the normal proton transfers are replaced with hydrogen-bonding interactions (Scheme 2). The initial one-electron reduction of VK_1 produces $\text{VK}_1^{\cdot-}$, which undergoes

Scheme 2. Electrochemical Reduction and Hydrogen-Bonding Mechanism for VK_1 in Aprotic Organic Solvents Containing Water^a



^a The counterion for the charged species is the supporting electrolyte cation, Bu_4N^+ .

little hydrogen bonding at water concentrations <0.5 M. As the water concentration increases above 0.5 M, $\text{VK}_1^{\cdot-}$ reacts to form the hydrogen-bonded species, $\text{VK}_1^{\cdot-}(\text{H}_2\text{O})_{a,b}$. The number of water molecules undergoing hydrogen bonding to the oxygen atoms increases with increasing water concentrations, according to an equilibrium between the hydrogen and non-hydrogen-bonded forms. Since the two oxygen atoms in VK_1 are nonequivalent, it is possible that the oxygen atoms undergo differing degrees of interaction with H_2O (hence the existence of two hydrogen-bonded forms: $\text{VK}_1^{\cdot-}(\text{H}_2\text{O})_a$ and $\text{VK}_1^{\cdot-}(\text{H}_2\text{O})_b$).

$\text{VK}_1^{\cdot-}$ (or $\text{VK}_1^{\cdot-}(\text{H}_2\text{O})_{a,b}$) can be further reduced by one electron to form VK_1^{2-} , which *immediately* undergoes hydrogen bonding, even at intermediate water concentrations (0.05 M). The electron-transfer step could occur consecutively (horizontal lines) or concertedly (diagonal lines) with the hydrogen bonding (Scheme 2).³⁴ The spectroscopic experiments indicate that the amount of hydrogen bonding is influenced by the concentration of water, and thus the UV–vis spectra display different extremes. At low water concentrations (≤ 0.05 M), $\text{VK}_1^{2-}(\text{H}_2\text{O})_{n,m}$ exists, whereas $\text{VK}_1^{2-}(\text{H}_2\text{O})_{x,y}$ exists at high water concentrations (≥ 5 M) with the H_2O coefficients $n,m < x,y$ (Scheme 2). An equilibrium exists between the hydrogen-bonded forms at intermediate water concentrations. A summary of the spectra of the different species is given in Figure 7, which were obtained under conditions that most favored their formation. A pure spectrum of $\text{VK}_1^{\cdot-}(\text{H}_2\text{O})_{a,b}$ was not obtained since it always existed in equilibrium with $\text{VK}_1^{\cdot-}$. The hydrogen bonding between water and the VK_1 anions is likely to involve a fluctuating network of flickering (not static) hydrogen bonds. Therefore, it is difficult to determine the exact number of water molecules involved in the interactions, but the numbers certainly vary depending on the water concentration as shown by how the voltammetry and UV–vis spectra vary as the water content is changed.

(34) (a) Costentin, C.; Robert, M.; Savéant, J.-M. *Chem. Phys.* **2006**, *324*, 40–56. (b) Costentin, C. *Chem. Rev.* **2008**, *108*, 2145–2179.

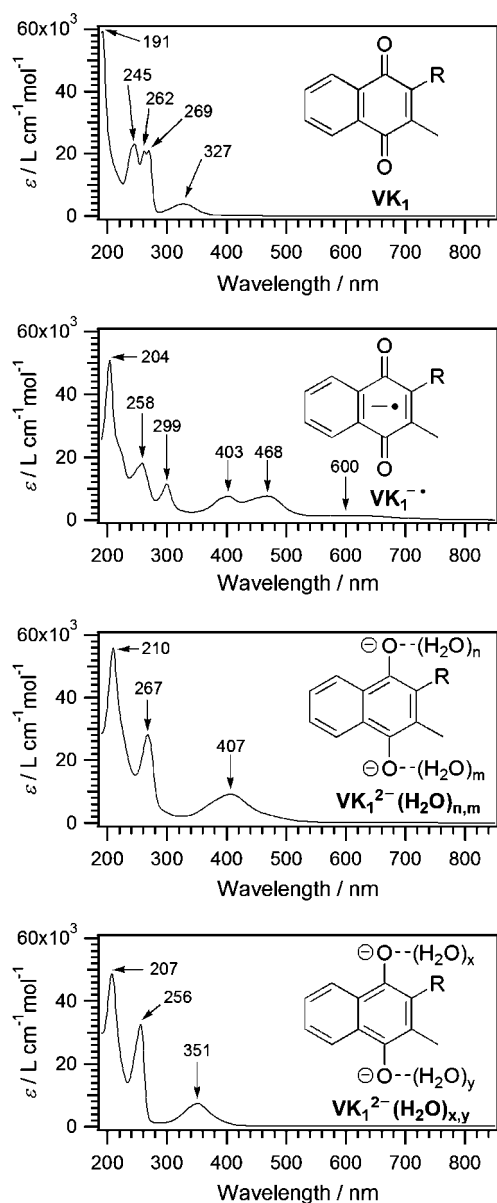


Figure 7. In situ UV-vis spectra obtained in an OSTLE cell during the reduction of 1 mM VK₁ in CH₃CN with 0.2 M Bu₄NPF₆ and varying concentrations of water. The UV-vis spectra of VK₁^{•-} and VK₁^{•2-}(H₂O)_{n,m} were obtained via the one- and two-electron respective reductions of VK₁ in the presence of 0.05 (±0.01) M H₂O. The UV-vis spectrum of VK₁^{•2-}(H₂O)_{x,y} was obtained via the two-electron reduction of VK₁ in the presence of 9.26 M H₂O.

To confirm that the reduction process produced the hydrogen-bonded species and not the protonated forms, electrolysis experiments were conducted at high water concentrations and the reduced species were then reacted with the strong non-aqueous acid, CF₃SO₃H. Figure 8a (black line) shows a CV of 1 mM VK₁ in CH₃CN containing 9.26 M H₂O. Figure 8a (red line) shows the CV obtained of the same solution after the VK₁ was reduced by two electrons per molecule under bulk controlled potential electrolysis conditions and the resulting dianion was reacted with CF₃SO₃H to form the hydroquinone (VK₁H₂) (eq 11).



The two voltammograms in Figure 8a differ in that the CV for VK₁ (black line) was initially scanned in the negative

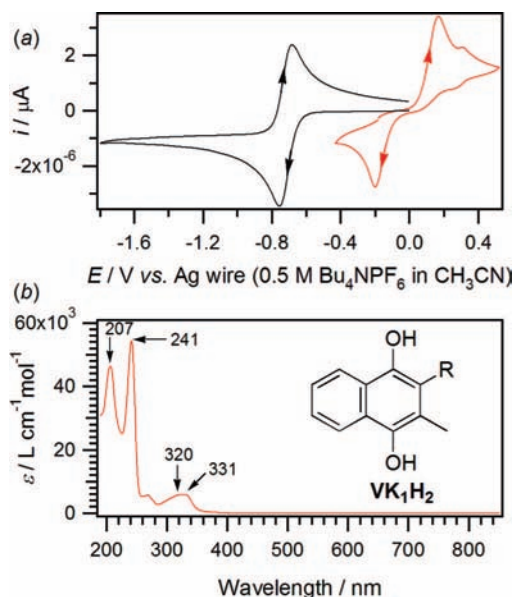


Figure 8. Electrochemical and spectroscopic data obtained during the electrolysis of 1 mM VK₁ in CH₃CN containing 0.2 M Bu₄NPF₆ and 9.26 M H₂O at 293 K. (a) Cyclic voltammograms obtained at a 1-mm diameter planar GC electrode. (Black line) Before the electrolysis of VK₁. (Red line) After the two-electron exhaustive reductive electrolysis of VK₁ (to form VK₁^{•2-}(H₂O)_{x,y}) and the addition of 0.05 M of CF₃SO₃H (to form VK₁H₂). (b) UV-vis spectrum of VK₁H₂ that was prepared by electrolyzing VK₁ and then adding CF₃SO₃H.

potential direction (reduction), while the CV for VK₁H₂ was initially scanned in the positive potential direction (oxidation). The voltammetry observed for VK₁H₂ is similar to that observed for other hydroquinones (such as dopamine) in acidic conditions; that is, they can be reversibly oxidized in a two-electron two-proton process with a wide separation between the forward and reverse peaks.^{3a,35} The minor additional processes detected at 0.2–0.3 V in the CV of VK₁H₂ are possibly associated with a small amount of an additional reaction product formed during the electrolysis/protonation reaction.

The UV-vis spectrum of VK₁H₂ is given in Figure 8b, which displays relatively strong and sharp bands at 207 and 241 nm and broader weaker bands at 320 and 331 nm. The spectrum is similar to that observed for the closely related 1,4-dihydroxynaphthalene.³⁶

3.3. Electrochemistry at Low (0.001–0.05 M) Water Concentrations. Having established the electrochemical and spectroscopic behavior of VK₁ in intermediate to high water concentrations, voltammetric experiments were next conducted at very low water concentrations by carefully drying the solvents and accurately measuring the water content by KF titration (see Experimental Section for details).

For water concentrations between 0.001 and 0.05 M, H₂O was allowed to increase under natural humidity conditions from the lowest level.²² Even under an argon atmosphere, the water content increased from 0.01 to 0.05 M within approximately 2 h for CH₃CN solutions. To have the initial water content below 0.05 M, it was necessary to use a pseudo-Pt wire as the reference electrode, located in the same compartment as the working

(35) (a) Hawley, M. D.; Tatawawadi, S. V.; Piekarski, S.; Adams, R. N. *J. Am. Chem. Soc.* **1967**, *89*, 447–450. (b) Sternson, A. W.; McCreery, R.; Feinberg, B.; Adams, R. N. *J. Electroanal. Chem.* **1973**, *46*, 313–321.

(36) Brahmia, O.; Richard, C. *Photochem. Photobiol. Sci.* **2003**, *2*, 1038–1043.

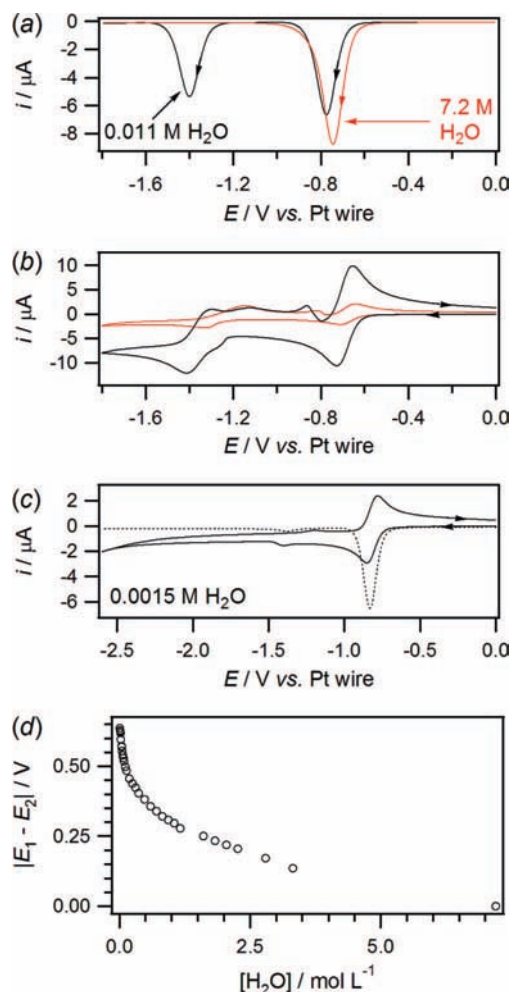


Figure 9. Voltammetric data recorded at a 1-mm diameter planar GC electrode in CH_3CN with 0.2 M Bu_4NPF_6 at 293 K. CVs were recorded at a scan rate of 0.1 V s^{-1} , and SWVs were recorded with a pulse period (τ) = 25 Hz, a potential step = 2 mV, and a pulse amplitude = 20 mV. (a) SWVs of 1.0 mM VK_1 with different concentrations of water. (b) CVs of (red line) 1.0 mM VK_1 and 0.005 (± 0.002) M H_2O and (black line) 5.0 mM VK_1 and 0.005 (± 0.002) M H_2O . (c) In situ (—) CV and (⋯) SWV obtained in a solution containing 1.4 mM VK_1 and 3 Å molecular sieves. (d) Plot of cathodic peak separations measured by SWV versus water content during the electrochemical reduction of 1.0 mM VK_1 .

electrode. Therefore, accurate potentials could not be obtained for the first and second processes; however, this was not considered problematic because the low concentration experiments were concerned with measuring the *potential difference* between the first and second processes and these potentials were unlikely to drift on the timescale of a voltammetric scan (a few seconds). Figure 9a shows a SWV of 1 mM of VK_1 in CH_3CN containing 0.011 (± 0.002) M water. At this concentration, the voltammogram shows two clear processes separated by 0.635 V. The second process was always smaller than the first process in SWV experiments because of relatively slow heterogeneous electron transfer.

Interesting voltammetric features were observed when the water concentration was below 0.010 M. Figure 9b (red line) is a CV obtained of 1 mM VK_1 in the presence of 0.005 (± 0.002) M H_2O . While the first process appears as a chemically reversible one-electron process, the second process has a wider than normal peak-to-peak separation (200 mV) with broad and nonideal shaped peaks, and with additional complicated features detected on the reverse oxidation sweep at $\sim -0.8 \text{ V}$ vs Pt wire.

The unusual peak shapes are possibly caused by interactions of the dianions with the electrode surface, because of the low concentrations of water reducing the amount of hydrogen bonding and lowering the solubility of the dianion. Figure 9b (black line) shows the CV of VK_1 at a higher concentration (5 mM) but in the presence of a lower water content ratio (0.005 (± 0.002) M). Similar features were observed to the lower concentration experiment with the second process showing additional features and an extra process evident at -0.8 V vs Pt wire when the scan direction was reversed.

Another feature that is evident in the voltammograms in Figure 9b is that the $|E_1 - E_2|$ values measured for a 5 mM solution of VK_1 are greater than those for a 1 mM solution of VK_1 , for close to equivalent H_2O concentrations ($|E_1 - E_2| = 680$ and 645 mV, respectively). This effect is only observable at concentrations of $\text{H}_2\text{O} < \sim 0.03 \text{ M}$, while at H_2O concentrations $> \sim 0.03 \text{ M}$, the $|E_1 - E_2|$ values are the same (within experimental error) for different concentrations of VK_1 in CH_3CN measured at equivalent concentrations of H_2O .

Experiments were also conducted at very low water concentrations by performing in situ voltammetric experiments in the presence of 3 Å molecular sieves. Under these conditions, it was possible to lower the water content to 0.0015 (± 0.0005) M, almost the same as that of VK_1 (Figure 9c). Interestingly, both the CV and SWV showed the first process, which seemed unchanged from other experiments, but the second process was difficult to detect. This result can be interpreted in two ways: (i) the electron transfer step occurs concertedly with the hydrogen bonding, so if there is insufficient water present the electron transfer does not occur, or (ii) the very low water content results in an unusual adsorptive effect where the reduction of the semiquinone is somehow masked. A small reductive process can be detected close to the potential where the semiquinone is normally reduced, but with a wide peak-to-peak separation (Figure 9c). In light of the results shown in Figure 9b, it is more likely that scenario (ii) occurs, so that the lack of the second process in very low water concentration environments is due to specific interactions of the dianion with the electrode surface.

The results in Figure 9b,c indicate that low levels of water make a large difference to the voltammetric behavior of VK_1 , especially the second reduction process. It is possible that some of the unusual voltammetric behavior reported for quinones in organic solvents can be explained by low levels of water in the solvent or by the effects of dissolved molecular oxygen as shown in Figure 3. Lehmann and Evans examined a number of quinones in aprotic media and observed anomalous voltammetric behavior, some of which was of unknown origin.^{19a} The CV for VK_1 in DMF (Figure 5) appears quite similar to that obtained for VK_1 in CH_3CN containing very low levels of water (Figure 9b). Therefore, the complicated voltammetry in DMF could be caused by a strong interaction of the dianion with the electrode surface.

On the basis of the electrochemical results on VK_1 , it seems appropriate that studies that report on the electrochemical behavior of quinones in non-aqueous solvents should also report the trace water content of the solvent to enable unambiguous comparisons with different data sets. This is particularly true for studies that examine interactions between reduced quinones and a range of hydrogen-bonding additives (such as alcohols and amines), because the trace water content of the solvent could significantly affect the measured $|E_1 - E_2|$ values.^{17,18} With this point in mind, we constructed calibration curves of the potential

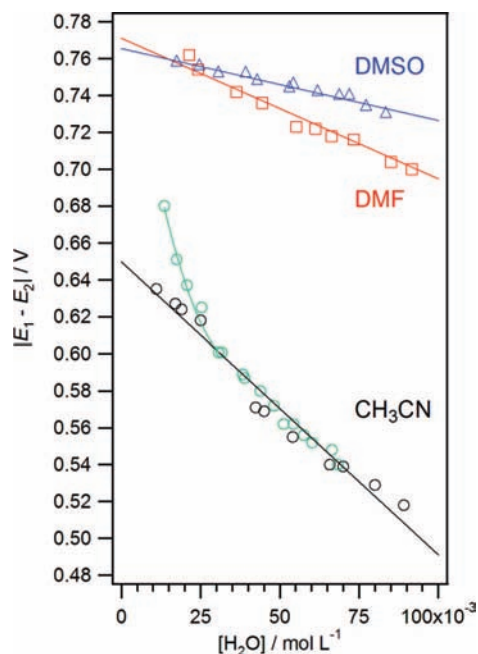


Figure 10. Plots of $|E_1 - E_2|$ measured by SWV during the reduction of 1.0 mM VK₁ at 293 K at a 1-mm diameter GC electrode, versus the water content of the solvent (CH₃CN, DMF, and DMSO, all containing 0.2 M Bu₄NPF₆) measured by KF titration. SWVs were recorded with a pulse period (τ) = 25 Hz, a potential step = 2 mV, and a pulse amplitude = 20 mV. The data points in green in CH₃CN were obtained with 5 mM VK₁.

separation between the first and second processes ($E_1 - E_2$) against the water content of the solvent, which allows a straightforward method for determining the concentration of water. Figure 9d shows the aforementioned plot over a wide water content range (0.01–9.26 M). The plot illustrates that there is an abrupt increase in the potential separation ($E_1 - E_2$) as the water content approaches zero. From an analytical perspective, the more useful range for measuring the water content is between 0 and 0.1 M, since it is straightforward to exclude water at higher concentrations and in many instances where “dry” solvents are reported the water content could still be in the millimolar range.

Figure 10 shows plots of the $|E_1 - E_2|$ separation (in volts) against the water content of the solvent between 0.01 and 0.1 M. In this range, the graphs are linear, which enabled straight lines to be fitted. The results were not reliable at water concentrations <0.01 M because of adsorption effects, resulting in the voltammetric peak shapes becoming complicated. The plots were constructed for three solvents, CH₃CN, DMSO, and DMF. For CH₂Cl₂, it was found that the KF method did not give consistent measurements for water content, possibly because of poor solubility of CH₂Cl₂ in the anode compartment of the KF titration cell (the solution appeared cloudy when CH₂Cl₂ was added).

The plots in Figure 10 are summarized in equation form in Table 1. The data in Table 1 provide a simple procedure to estimate the water content of the solvent by measuring the $|E_1 - E_2|$ values and applying eq 12. It is preferable that the VK₁ concentration is close to 1 mM to avoid concentration-dependent deviations of the straight lines at very low water concentrations (as shown in Figure 9b and 10 for data obtained in CH₃CN). The uncompensated solution resistance is not expected to reduce the accuracy of the measured $|E_1 - E_2|$ values because each individual potential will be uniformly affected. Furthermore, providing that low scan rates are used for the CV or SWV

Table 1. Parameters That Can Be Used To Estimate the Water Content of a Solvent between 0.01 and 0.1 M According to the Equation $\text{Water Content (mol L}^{-1}\text{)} = (a \times |E_1 - E_2|) + b^a$

solvent	a/mV^{-1}	b/M	usable range $ E_1 - E_2 /\text{V}$	$a^{-1}/\text{mV mM}^{-1}$
CH ₃ CN	-0.611	0.399	0.490–0.640	-1.64
DMF	-1.274	0.984	0.695–0.765	-0.78
DMSO	-2.462	1.886	0.725–0.762	-0.41

^a The concentration of VK₁ used for obtaining $|E_1 - E_2|$ values should equal 1.0 ± 0.2 mM.

experiments, there will be minimal distortion of the voltammograms due to resistance effects.

$$\text{water content (mol L}^{-1}\text{)} = (a \times |E_1 - E_2|) + b \quad (12)$$

There are substantial differences in the slopes of the plots for the different solvents. The parameter a^{-1} in Table 1 is the change in $|E_1 - E_2|$ for every 1 mM change in water concentration. The largest change in the $|E_1 - E_2|$ values is observed in CH₃CN, which decreases by -1.6 mV/1 mM increase in water, compared to that in DMSO where the $|E_1 - E_2|$ values decrease by -0.4 mV/1 mM increase in water (between 0.01 and 0.1 M water). $|E_1 - E_2|$ values below the usable range in Table 1 indicate that the solvent is wetter than 0.1 M, whereas $|E_1 - E_2|$ values above the usable range indicate that the solvent is extremely dry ($\sim < 0.01$ M water).

The primary error in measuring the water content in this study is systematic and arises from transferring a sample of the test solution from the electrochemical cell into the KF titration cell. During the transfer, it is likely that some atmospheric water will enter the syringe needle, but this is only avoidable if both reactions are performed in a dry box. However, the observation that there is not an excessive scatter in the slopes of the plots in Figure 10 would suggest that the systematic error is not severe. This is because the data in Figure 10 are a combination of a least three experimental runs for each solvent, under conditions where the atmospheric humidity varied, but there is no obvious change in slope between the different experiments. With the low water concentration measurements it was not possible to perform more than one measurement at a fixed water concentration (to obtain a statistical average) because the water content was continually changing over time.

It is envisaged that the procedure developed in this study will be of primary use to electrochemists as a convenient method of testing the approximate water content of their solvent. As well as for quinone electrochemistry, the data in Table 1 will be useful in any situation where low levels of water are known to influence the voltammetric behavior, such as for hydrolysis reactions that occur during the oxidation of vitamin E.³⁷ Disadvantages of using the KF titration directly are that it cannot be performed in situ and the reagents are expensive and require frequent replenishment. Therefore, in a similar way that ferrocene is often added to non-aqueous solvents as an internal calibrant for the formal potential, VK₁ (or another quinone after

(37) (a) Williams, L. L.; Webster, R. D. *J. Am. Chem. Soc.* **2004**, *126*, 12441–12450. (b) Lee, S. B.; Lin, C. Y.; Gill, P. M. W.; Webster, R. D. *J. Org. Chem.* **2005**, *70*, 10466–10473. (c) Wilson, G. J.; Lin, C. Y.; Webster, R. D. *J. Phys. Chem. B* **2006**, *110*, 11540–11548. (d) Lee, S. B.; Willis, A. C.; Webster, R. D. *J. Am. Chem. Soc.* **2006**, *128*, 9332–9333. (e) Webster, R. D. *Acc. Chem. Res.* **2007**, *40*, 251–257. (f) Peng, H. M.; Webster, R. D. *J. Org. Chem.* **2008**, *73*, 2169–2175. (g) Yao, W. W.; Peng, H. M.; Webster, R. D. *J. Phys. Chem. B* **2008**, *112*, 6847–6855.

calibration studies) can be added as a simple test for the amount of trace water in CH₃CN, DMF, and DMSO.

4. Conclusions

This work has demonstrated that differences of a few millimolar water can have a pronounced effect on the reductive electrochemistry of a quinone in non-aqueous solvents, especially at low water levels (<0.05 M). Therefore, it is important that electrochemical studies on quinones in non-aqueous solvents report the initial water content of their solvents, since trace amounts of water are likely to interfere with measurements made in the presence of other hydrogen-bonding donors (such as alcohols and amines). A procedure was developed for approximating the water content of the solvents CH₃CN, DMSO, and DMF between 0.01 and 0.1 M H₂O, irrespective of the reference electrode used, by performing a single cyclic or square-wave voltammogram in the presence of 1 mM VK₁ to obtain the $|E_1 - E_2|$ value, and then applying eq 12 and the parameters in Table 1.

The voltammograms and UV–vis spectra obtained during the one- and two-electron reduction of VK₁ in CH₃CN in the presence of varying H₂O concentrations allowed estimations of the degree of hydrogen bonding. The semiquinone anion radical exists in a predominantly non-hydrogen-bonded form at concentrations of water <0.5 M, since the UV–vis spectra and

the voltammetric potential (E_1) changed by a small amount when the water concentration was varied below this level. In contrast, the quinone dianion undergoes extensive hydrogen bonding at even very low water concentrations, indicated by how the second voltammetric peak (E_2) shifts substantially over a wide range of water concentration (0.01–7.2 M) and by how the bands in the UV–vis spectra shift uniformly with water concentrations between 0.05 and 5 M. It is unlikely that the UV–vis spectrum of the non-hydrogen-bonded dianion can be obtained unless extremely dry solvents can be used, where $[H_2O] \leq [VK_1]$. The voltammetric behavior became very complicated when the concentration of water began to approach that of the substrate. At these very low water concentrations ($[H_2O] < 0.01$ M), there is insufficient water for favored hydrogen bonding of the dianion, likely resulting in a decrease in solubility of VK₁²⁻ and hence the appearance of adsorptive processes in the voltammograms.

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Note Added after ASAP Publication. The version of this paper published on January 8, 2009, contained an error in the abstract. The version published on January 13, 2009 has the correct information.

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