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Electrochemical and Spectroelectrochemical Behaviors of Vitamin K_1/Lipid Modified Electrodes and the Formation of Radical Anion in Aqueous Media†

JeeEun Yang,‡ Jang-Hee Yoon, Mi-Sook Won,* and Yoon-Bo Shim‡,*

*Busan Center, Korea Basic Science Institute, Busan 609-735, Korea. *E-mail: mswon@kbsi.re.kr*
*‡Department of Chemistry, Pusan National University, Busan 609-735, Korea. *E-mail: ybshim@pusan.ac.kr*
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The electrochemical properties of the liposoluble vitamin K_1 adsorbed on bare and lipid coated glassy carbon electrodes (GCEs) were studied in unbuffered and well buffered aqueous media. The reduction products of vitamin K_1 were characterized by employing cyclic voltammetry and the *in situ* UV-visible spectroelectrochemical technique. The radical species of vitamin K_1 cannot be observed at the bare GCEs in well buffered media. The formation of the anion radical of vitamin K_1 was observed in unbuffered solutions above pH 5.9 or at the lipid coated GCE in a well-buffered solution. UV-visible absorption bands of neutral vitamin K_1 were observed at 260 nm and 330 nm, and a band corresponding to the anion radical species was observed at 450 nm. The derivative cyclic voltabsorptometric (DCVA) curves obtained for electrochemical reduction of vitamin K_1 confirmed the presence of both neutral and anion radical species. The anion radical of vitamin K_1 formed at the hydrophobic conditions with phosphatidylcholine (PC) lipid coated electrode was stable enough to be observed in the spectroelectrochemical experiments.

Key Words: Vitamin K_1, Electron transfer reaction, *In situ* UV-visible, Anion radical, Lipid

Introduction

Elucidating the electron transfer mechanism of redox processes of riboflavin, quinones, cytochrome and NADH[1-4] in the cell membrane is essential because they generated ATP by the electron transfer through the cell membrane. Of these, quinones are one of the most important and well-studied examples of organic redox couples.[5-6] They are well-represented examples of the electron transfer processes of the biological species in the photosynthetic reaction center and in mitochondrial ATP synthesis.[7] We have also studied the electrochemical behavior and anion radical detection of benzoquinone.[8-10]

Vitamin K has the chemical structure based on 2-methyl-1,4-naphthoquinone derivatives with an aliphatic side chain in the 3-position and it is involved in cellular respiration and in oxidative phosphorylation[11] as electron carriers. In addition, vitamin K functions as a blood clotting cofactor[12] and participates in bone mass increase.[13] For these reasons, knowledge of the redox and the electron transport properties of vitamin K are important for a better understanding of their behavior in biological environments. To observe electron transport properties of vitamin K, studies have been carried out employing polarography[14] and cyclic voltammetry[15-16] in aqueous and non aqueous media.[17-18] The electrochemical behavior of vitamin K has been investigated using various modified electrodes, such as gold[19] and platinum electrodes modified with cysteamine, cystamine[20] *n*-alkanethiols,[21] mercury-coated carbon fiber microelectrode,[18] interdigitized array microelectrode,[22] *etc*. Few reports, however, have been reported for the electrochemical properties of vitamin K in lipid membrane systems. Although electrochemical behavior of vitamin K was investigated in various conditions, there is no report for the formation of anion radical as an intermediate in aqueous media by electrochemical techniques.

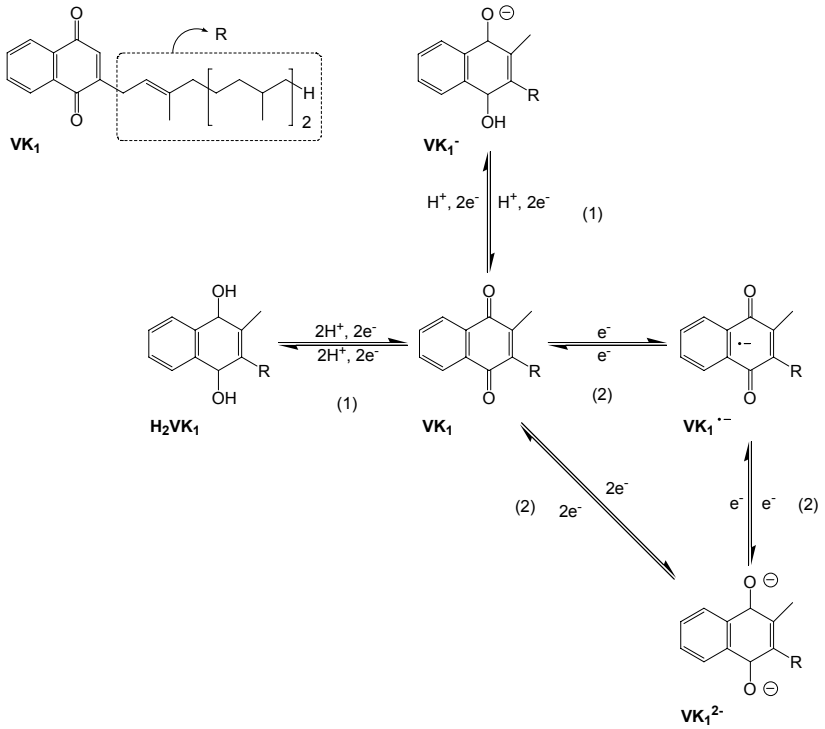
In the present study, the electrochemical behavior of vitamin K_1 (VK_1) adsorbed on a bare and VK_1/PC coated electrodes was investigated in unbuffered and well buffered aqueous media. The stability of vitamin K_1 anion radical as an intermediate species was studied in unbuffered aqueous media and at the lipid coated GCE in well-buffered solutions. The lipid used in this work, PC, had both ammonium and phosphoric acid as a head group. The effects of the pH and composition of the electrolyte solution on the redox reaction of VK_1 was evaluated by employing cyclic voltammetry and *in situ* UV-visible spectroelectrochemical techniques.

Experimental

Reagents and solutions. Vitamin K_1 (VK_1) and phosphatidylcholine (PC) were purchased from Sigma-Aldrich Co (USA) and Avanti Polar Lipids Inc, respectively. VK_1/PC solutions were dissolved in a chloroform solution to an adequate concentration. All aqueous solutions were prepared in doubly distilled water obtained from a Milli-Q water-purifying system (18 MΩ cm). Well-buffered aqueous solutions in various pHs were prepared using the mixtures of HCl/potassium chloride and citric acid/NaH_2PO_4. The unbuffered aqueous solutions were prepared by adjusting a 0.1 M H_2SO_4 solution to the desired pH by adding proper amounts of a 0.1 M NaOH solution.

Instruments. Before each experiment, the GC electrode was polished with 0.1 μm alumina powder to a mirror finish and rinsed with ethanol. The electrode potentials were measured with respect to an Ag/AgCl (sat'd KCl) electrode. The counter electrode was a Pt wire. The temperature of the solution was 25 °C. Cyclic voltammetry (CV) was performed using a potentiostat/galvanostat, Kosentech Model PT-2 (S. Korea). The *in situ* UV-visible spectra were obtained from the assembly of a

†This paper is dedicated to Professor Hasuck Kim for his outstanding contribution to electrochemistry and analytical chemistry.



Scheme 1. Electrochemical redox mechanism for VK_1 in the various well-buffered solutions (1) and unbuffered solutions (2).

CCD detector, a Xenon flash lamp and a bifurcated optical fiber made by Ocean Optics Co. An electrochemical cell with a quartz window was used for the *in situ* experiment. The UV-Visible absorption spectra and DCVA curve were obtained using the previously reported method.[23-24] A glassy carbon (GC) electrode was modified with VK_1 or VK_1/PC by spin coating at 250 rpm to increase homogeneity of the casting layer of VK_1 or VK_1/PC.

Results and Discussion

Electrochemical characterization of the VK_1 modified electrode. As shown in Figure 1(A), CVs were recorded for a VK_1 coated electrode in various well-buffered solutions at different pHs. Redox pairs of VK_1 in CVs recorded at the pHs 1.0, 3.0, 5.0, 7.0, and 9.0 appeared at 286/−281, 213/−347, 93/−435, −7/−509, and −177/−579 mV (E_{pa}/E_{pc}), respectively. The quarsi-reversible redox peak of VK_1 shifted to a more positive potential as the pH decreased. ΔE_p of each redox pair was 567, 560, 528, 502 and 402 mV, respectively. The reversibility of VK_1 redox peak was better at higher pHs. Figure 1(B) shows the peak potential (E) as a function of pH ranged from 1.0 to 10.0 (well-buffered solutions). In this diagram, the degree of oxidation and protonation of VK_1 could be obtained from the dE^0/dpH values by using the Nernst equation.[2,6] Where E^0 is a (Epa + Epc)/2. This result, we observed two different kinds of slopes. The redox process of VK_1 is expected as follows; (1) in the range of $1.0 \le pH \le 9.5$, $dE^0/dpH = -56$ mV leads to the formation of H_2VK_1 by the reaction of VK_1 with two protons ($2H^+$) and two electrons ($2e^-$). In the range of $pH \ge 9.5$, $dE^0/dpH \approx 0$ mV can be explained by the two redox process of (2) and (3). However, according to our previous work,[8] the redox process of VK_1 was reported to be proceed with the formation of VK_1^{2-} by the reaction of VK_1 with two electrons ($2e^-$) (2).

$$VK_1 + 2H^+ + 2e^- \leftrightarrow H_2VK_1 \quad (1)$$

$$VK_1 + 2e^- \leftrightarrow VK_1^{2-} \quad (2)$$

$$VK_1 + e^- \leftrightarrow VK_1^{-\bullet} \quad (3)$$

The observation of quinone anion radical in the well-buffered aqueous solution was not possible because the anion radical reacted very quickly with the proton (H^+), owing to unstability of the anion radical produced through the electrochemical reduction of quinones in aqueous media.[2,8-10] For observation of anion radical in the well-buffered aqueous solution, we have studied the redox reaction of VK_1 with PC (VK_1/PC). Figure 1(C) shows CVs recorded for a VK_1/PC modified electrode in various pHs of well-buffered solutions. Redox pairs of VK_1/PC in CVs recorded at the pHs 1.0, 3.0, 5.0, 7.0 and 9.0 appeared at 265/−286, 205/−349, 120/−445, 8/−518 and −43/−605 mV (E_{pa}/E_{pc}), respectively. The redox peak of VK_1 shifted to a more positive potential as the pH decreased. Figure 1(D) shows the E-pH diagram of VK_1/PC modified electrode in well-buffered solutions. The mechanism is expected as follows: ‹1› in the range of $1.0 \le pH \le 9.0$, $dE^0/dpH = -34$ mV leads to the formation of HVK_1^- by the reaction of VK_1 with one proton (H^+) and two electrons ($2e^-$), and ‹2› in the range of $pH \ge 9.0$, $dE^0/dpH \approx 0$ mV can expect to formation of $VK_1^{-\bullet}$ by the reduction of VK_1 through the one electron (e^-) transfer reaction.

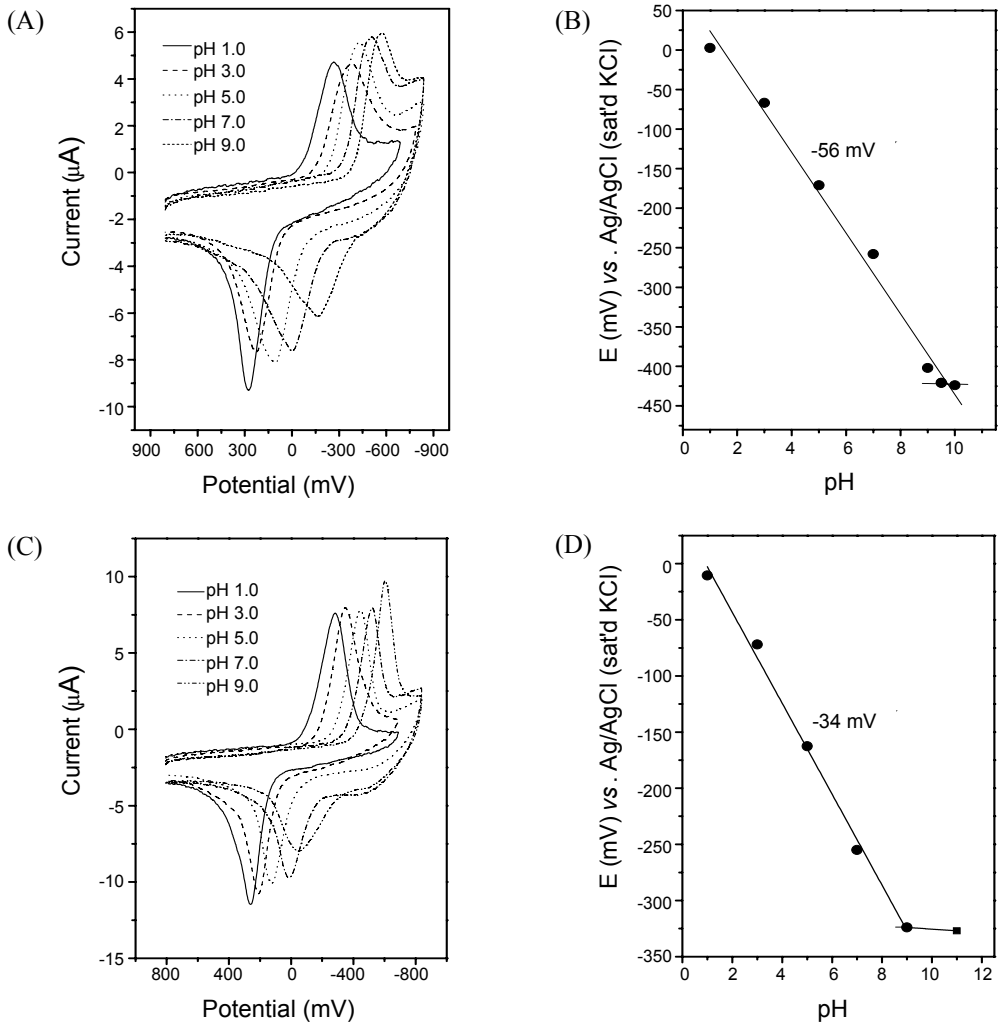


Figure 1. (A) The cyclic voltammograms recorded for the VK_1 modified electrode at various pH in well-buffered solutions. (B) The E-pH diagram obtained for the VK_1 modified electrode. (C) The cyclic voltammograms recorded for the VK_1/lipid modified electrode at various pH values in well-buffered solutions. (D) The E-pH diagram obtained for the VK_1/lipid modified electrode.

$$VK_1 + H^+ + 2e^- \leftrightarrow HVK_1^- \quad \langle 1 \rangle$$

$$VK_1 + e^- \leftrightarrow VK_1^{-\bullet} \quad \langle 2 \rangle$$

Unlike the mere VK_1 coated-electrode, the formation of quinone anion radical at the VK_1/PC modified electrode can be evaluated from the E *vs*. pH graph. Figure 2(A) shows the CVs recorded at various pHs in unbuffered solutions, which can be summarized as; (1) a single pair of redox peaks (peak I and I') (155/−321 mV, E_p = 476 mV) appeared at pHs lower than about 4.7. This pair (1) showed the same trend in a well buffered solution (VK_1/H_2VK_1). This means that the anion radical of vitamin K ($VK_1^{-\bullet}$) was not stabilized in this pH range; (2) two pairs of peaks were observed between about pH 4.7 and below pH 5.3; one (peak I and I') from the same origin as the redox peaks described in (1) (VK_1/H_2VK_1) and a new one (peak II and II') at a more negative potential than that for the first peak (pH 6.7; 72 mV/−329 mV/−582 mV). The new peaks II and II' could be attributed to the formation of $VK1^{-\bullet}$. The currents of peaks II and II' were increased and those of peaks I and I' decreased according to increasing pH; (3) a single pair of peaks from the same origin as a new one (peak II and II') in (2) was observed in the pH range higher than 5.0. As shown in Figure 2(B), a plot of E vs pH yielded two different slopes between pH 2.0 and 8.9. At $5.3 \leq pH \leq 5.9$, a plot of E vs pH yielded a slope of −28 mV/pH unit. This result was related to the formation of HVK_1^- by the reaction of $1H^+$ and $2e^-$ to VK_1. Above pH 5.9, the product will be $VK_1^{-\bullet}$, meaning the reaction involves 0 H^+, 1e^- and the slope of the E *vs*. pH plot becomes 0 mV/pH. Based on the electrochemical evidences shown herein, it was possible to propose the mechanism shown in the VK_1 electrochemical redox process in the well-buffered solutions and unbuffered solutions. It can be summarized as follows;

$$VK_1 + H^+ + 2e^- \leftrightarrow HVK_1^-$$

$$VK_1 + e^- \leftrightarrow VK_1^{-\bullet}$$

***In situ* UV-visible spectroelectrochemical studies.** The previous results showed the formation of reduction intermediates

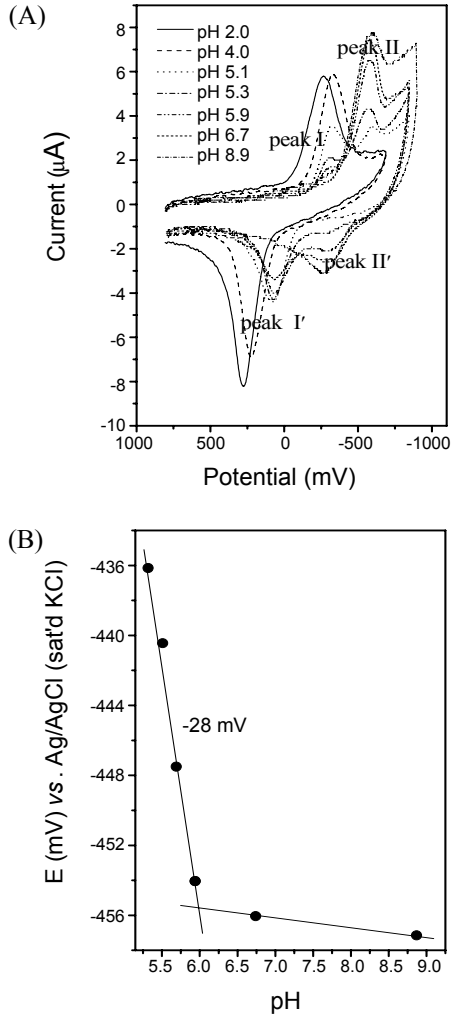


Figure 2. (A) The cyclic voltammograms recorded for the 1.0 mM VK_1 modified electrode in unbuffered solutions containing 0.1 M KCl. (B) The E-pH diagram obtained for the 1.0 mM VK_1 modified electrode in unbuffered solutions, which corresponded to the redox reaction of the peaks II and II'.

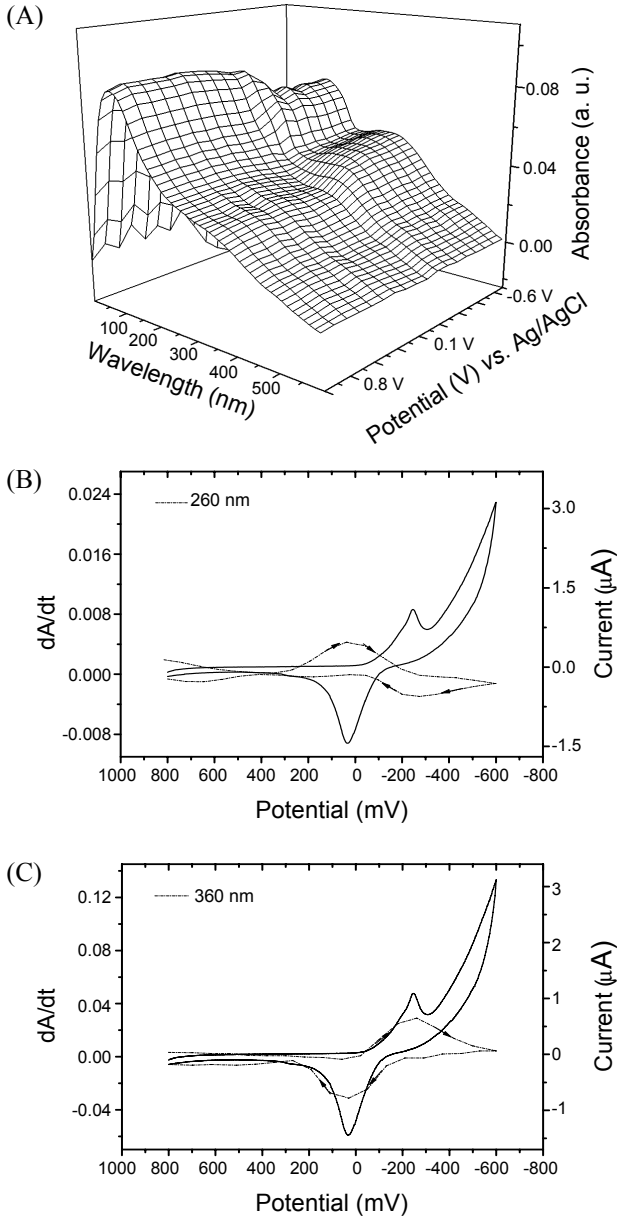


Figure 3. (A) *In situ* UV-visible spectra obtained for 1.0 mM VK_1 in well-buffered solutions (pH 3.0). (B) The DCVA curves at 260 nm obtained for VK_1 in the well-buffered solution (pH 3.0). The scan rate was 5 mV/sec. (C) The DCVA curves at 330 nm obtained for VK_1 in the well-buffered solution (pH 3.0). The scan rate was 5 mV/sec.

of VK_1 in well-buffered and unbuffered solutions. The *in situ* UV-visible spectroelectrochemical measurements were carried out to confirm the formation of VK_1 intermediates. The *in situ* UV-visible spectra obtained in well buffered solutions (Figure 3 (A)) showed that an absorption band assigned to H_2VK_1 appeared at 330 nm, along with another absorption band at 260 nm that corresponded to neutral VK_1. Upon the reduction of VK_1 at −0.6 V, the absorbance at 260 nm decreased, while a band at 330 nm increased, which was related to H_2VK_1 in the protonated form. To confirm the absorption band corresponding to the redox process of chemical species, the derivative cyclic voltabsorptometric (DCVA) experiment was performed. In the DCVA experiments, the derivative absorbance signal (dA/dt) was recorded at a given wavelength as a function of the potential. The DCVA curves recorded at 260 nm and 330 nm are shown in Figure 3(B) and (C), along with the concurrent CV. As can be seen in Figure 3(B) and (C), the DCVA curve showed the same redox potential obtained with the CV recorded in the well-buffered solution (pH 3.0), where H_2VK_1 produced by the reduction reaction of VK_1 through two electrons and two protons transfer are observed. Thus, this indicates that no other intermediates on the spectroelectrochemcial experimental timescale were observed in the well-buffered solution.

To obtain the direct evidence of the anion radical formation in unbuffered solutions, we ran spectroelectrochemical experiments. Figure 4(A) shows *in situ* UV-visible spectra obtained for 1.0 mM VK_1 at pH 6.7. The absorption bands at 330 nm and 260 nm showed spectra in the same fashion upon the reduction of VK_1 in the well-buffered solutions. Therefore, the bands at 260 nm and 330 nm were due to the absorption of photons by VK_1 and H_2VK_1, respectively. Unlike well-buffer-

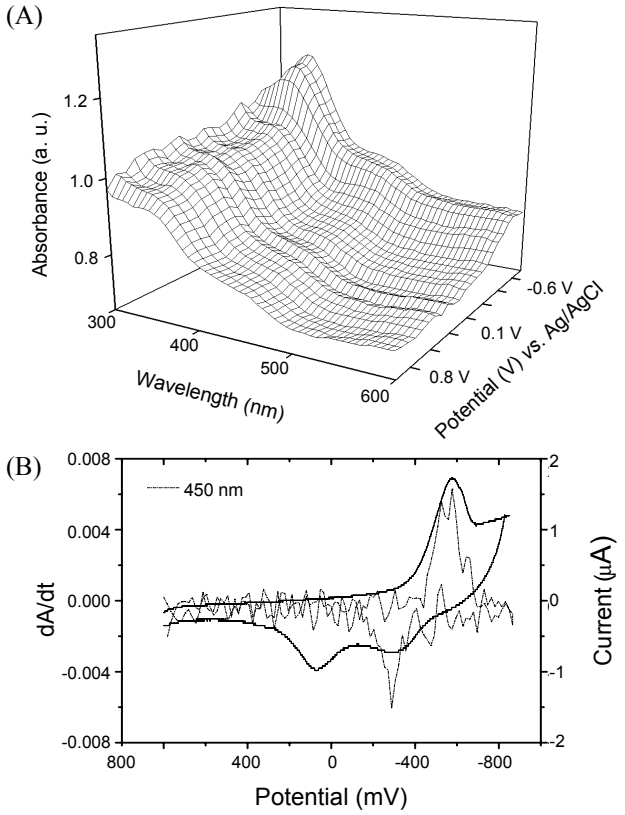


Figure 4. (A) *In situ* UV-visible spectra recorded for 1.0 mM VK_1 in unbuffered solutions (pH 6.7). (B) The DCVA curves obtained for VK_1 at 450 nm in the unbuffered solutions (pH 6.7). The scan rate was 5 mV/sec.

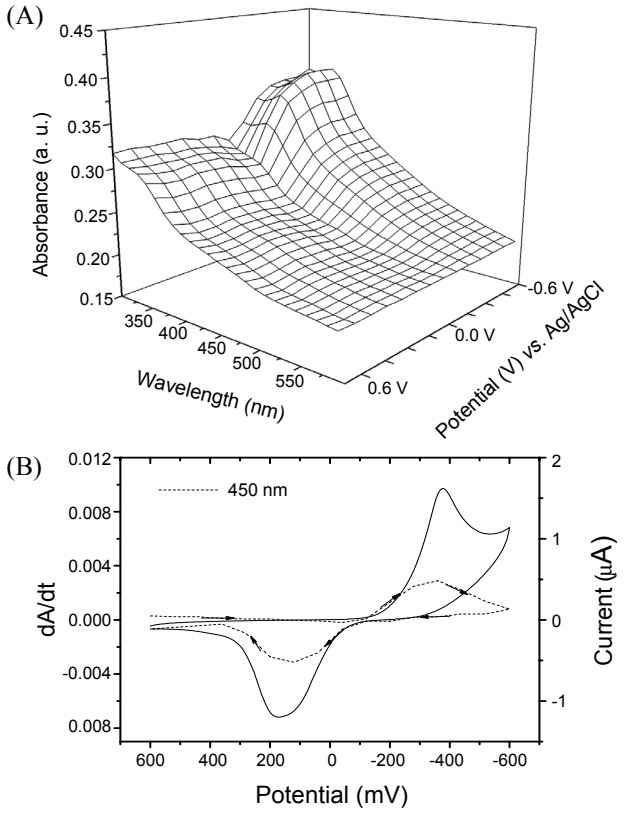


Figure 5. *In situ* UV-visible spectra nm obtained for VK_1/lipid in well-buffered solutions (pH 3.0). (B) The DCVA curves obtained for VK_1/lipid at 450 nm in the well-buffered solutions (pH 3.0). The scan rate was 5 mV/sec.

ed solutions, a new absorption band (450 nm) appeared in the unbuffered solution. As can be seen in Figure 4(B), the DCVA signal at 450 nm appeared and disappeared reversibly upon the reduction and re-oxidation of VK_1 at the potential range between +0.8 and −0.6 V in the unbuffered solution. The absorption band at 450 nm appeared due to the anion radical formed by the electrochemical reduction. Thus, it was confirmed that the band at 450 nm corresponded to the absorption of photon by $VK_1^{-\bullet}$ species. The DCVA curve at 450 nm was almost the same as the CV (peak II) observed in the unbuffered solution (pH 6.7). The matching DCVA and CV curves indicate that the species reduced at the peak potential of VK_1 was the anion radical form, where it was stable enough to be observed in the unbuffered solutions. It was clearly shown that the radical anion of VK_1 formed in the unbuffered solution above pH 5.9. The absorption band was similar to that of the VK_1 reduction product observed in a non-aqueous solution, which was shifted as much as 50 nm toward a longer wavelength.[25]

Figure 5 shows *in situ* UV-visible spectra obtained for VK_1/PC in the well-buffered solution (pH 3.0). Similarly in unbuffered solutions, a new absorption band (450 nm) appeared at lipid coated electrode even in the well-buffered solutions. In the case of using lipid (VK_1/PC), *in situ* UV-visible spectroelectrochemical experiments in well buffered solutions can confirm the intermediate of VK_1 reduction reaction. The anion radical form was stable enough to be observed in the unbuffered solutions and at lipid coated electrode in well-buffered solutions.

Conclusion

The electrochemical reduction of VK_1 was conducted by a two-electrons and two-protons transfer process in well-buffered solutions at all pH ranges. The observation of $VK_1^{-\bullet}$ was not possible in the well-buffered solutions because $VK_1^{-\bullet}$ reacts very quickly with proton ion (H^+) in the present experimental condition. On the other hand, VK_1 was easily reduced to H_2VK_1 at pHs lower than 5.0 in an unbuffered solution as follows; $VK_1 + 2H^+ + 2e^- \leftrightarrow H_2VK_1$. The predominant reactions was $VK_1 + H^+ + 2e^- \leftrightarrow HVK_1^-$ at $5.3 \leq pH \leq 5.9$ and $VK_1 + e^- \leftrightarrow VK_1^{-\bullet}$ at $pH \geq 5.9$. It is clearly shown that the electrochemical reduction of VK_1 proceeds to the formation of the anion radical in unbuffered solutions around pH 5.9 or more above and at the lipid coated electrode in well-buffered solutions.

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