Simultaneous EQCM and fluorescence detection of adsorption/desorption and oxidation for pyridoxol in aqueous KOH on a gold electrode

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Abstract

A simultaneous EQCM–fluorescence method has been proposed as a novel and informative tool for the study of the adsorption/desorption and oxidation for vitamin B₆ (pyridoxol) at a polycrystalline Au electrode in aqueous KOH. The oxidation of pyridoxol was irreversible and accompanied by a strong electrogenerated fluorescence emission peaking at 443 nm when excited at 360 nm. The adsorption and desorption of the oxidation product were found in the double layer region and were discussed by analyzing the simultaneous fluorescence and EQCM data. In addition, the voltammetrically electrogenerated fluorescence is proposed as a new and sensitive method for assay of pyridoxol with satisfactory results.

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Keywords: Simultaneous EQCM-fluorescence study; Pyridoxol oxidation; Au electrode; Alkaline solution; Quantitative analysis

1. Introduction

Pyridoxol, also known as VB₆, belongs to the vitamin family, being one of the indispensable organic compounds for normal metabolism in living systems. If lacking in VB₆, the human body may suffer from nervous excitation, depression, or vomiting. It is generally used as a drug for the treatment of sickness in pregnancy, neuritis, etc. Many methods, including differential pulse polarography [1], voltammetry [2,3], flow injection analysis [4], spectrophotometry [5,6], high-performance liquid chromatography [7] and capillary electrophoresis [8], have been used for property characterization and assay of pyridoxol.

A conventional electrochemical experiment generally records pure electrical parameters, namely, potential, current, impedance, electric charge, etc., to elucidate the electrochemical reaction mechanism or to deal with quantitative analysis problems. The addition of other parameters from simultaneous spectral measurements and quartz crystal microbalance (QCM) characterization can provide absolute complementary and real-time information, thus leading to less ambiguity in the description of a practical electrochemical process. Several groups have developed such an integration of research methods. Gottesfeld and co-workers [9] combined ellipsometry and electrochemical quartz crystal microbalance (EQCM) studies to monitor the optical properties of an electrogenerated polymer as a function of its thickness (or mass). Xie et al. [10] coupled reflectance spectroscopy and the EQCM to examine the reduction of an ammonium copper complex in the solution phase. Shimazu et al. [11] succeeded in producing sufficiently thin electrodes to investigate in the transmission mode the optical and gravimetric changes accompanying the redox transition of the species absorbed from the solution phase. Mo et al. [12] used normalized optical reflectivity measurements to study the absorption of bromide on an optically polished EQCM gold electrode. Xu et al. [13] introduced a beam of UV–Vis light via a bifurcated optical fiber to an
EQCM electrode for characterization of poly(1-naphthylamine) film growth, and a similar methodology with full spectral information was reported recently by Shim et al. [14]. Henderson and co-workers [15–18] have demonstrated the combination of EQCM with probe beam deflection (PBD) for temporal discrimination of ion and solvent transfer in some electrochemical processes. These integrated methods may also provide multi-mode selectivity simultaneously achievable in a single device for chemical analysis [19].

In this work, a simultaneous QCM–fluorescence–electrochemistry method has been developed as a novel and informative tool to monitor the electro-oxidation process of pyridoxol on a polycrystalline Au electrode in alkaline aqueous solutions, by which the simultaneous recording of QCM frequency and resistance, fluorescence and electrochemistry data is feasible. In addition, the voltammetrically electrogenerated fluorescence is proposed as a new protocol for assay of pyridoxol.

2. Experimental

2.1. Apparatus and reagents

The experimental setup is illustrated schematically in Fig. 1. Electrochemical experiments were performed on a CHI660A electrochemical workstation (CH Instruments Co., USA) controlled by the CHI660A software. Fluorescence measurements were carried out on an F-4500 fluorescence spectrophotometer (Hitachi Co., Japan) controlled by Hitachi FL Solutions software. A research quartz crystal microbalance (Maxtek Inc., USA) controlled by Maxtek RQCM software was used to record QCM frequency and resistance data. The research quartz crystal microbalance (RQCM) is able to record the resonant frequency \( f_0 \) and the motional resistance \( R_1 \) of the crystal accurately via its high performance phase lock oscillator circuit, as given in the operation manual provided by the manufacturer. Also, good agreement of the \( f_0 \) and \( R_1 \) responses to the increase of sucrose concentration, with results obtained by using the RQCM and an HP4395A impedance analyzer [20], was obtained after careful experimentation in this laboratory. Analog signals from the CHI660A and F-4500 were imported into the input channels of the RQCM for analog/digital conversion and subsequently synchronous digital recording of electrochemistry, fluorescence, and QCM data. UV–Vis spectra were collected on a TU-1221 UV–Vis spectrophotometer (PUXI General Co., China). AT-cut piezoelectric quartz crystals (PQCs) with a nominal frequency of 9 MHz and Au electrodes of 0.6 cm diameter were used. The PQC of 1.25 cm diameter was adapted and then sealed into the edge of a hole at a Teflon base using 704 silicon rubber adhesive. One PQC gold electrode (OE1) was in contact with the solution and served as the working electrode (WE), while the PQC gold electrode on the other side (OE2) was located in the waterproof air compartment, as shown in Fig. 1(b). The reference electrode (RE) was a saturated KCl calomel electrode (SCE) and all potentials are reported versus the SCE in this work. The auxiliary electrode (AE) was a platinum sheet. Pyridoxol hydrochloride was purchased from Sigma and used as received. All other reagents were of analytical grade or better. All solutions were prepared fresh prior to use, and twice-distilled water was used throughout. All experiments were carried out at room temperature (20 ± 2 °C).

2.2. Procedures

To remove possible surface contamination, the Au working electrode was carefully treated prior to use.
The gold electrode surface was treated with one drop of concentrated HNO₃ for ca. 15 s, then washed with twice-distilled water and dried via clean air blowing. The HNO₃ treatment was repeated three times, followed by a similar concentrated H₂SO₄ treatment for three times. The acid-treated Au electrode was then subject to potential cycling between 0 and 1.5 V (50 mV s⁻¹) in 0.2 mol l⁻¹ HClO₄ for sufficient cycles to obtain reproducible cyclic voltammograms and also QCM frequency and resistance responses [21,22]. The electrode was rinsed with twice-distilled water after completion of potential cycling.

The WE, RE and AE were placed in the spectroelectrochemical cell which was inserted into the 1 cm cuvette holder of the F-4500, as shown in Fig. 1, then a test solution was injected into the cell to immerse the electrode surfaces. Electrochemistry, fluorescence, frequency, and resistance responses were monitored simultaneously during multi-mode experiments. The sample drug solution for assay of pyridoxol was prepared by dissolving one tablet of pyridoxol in 0.10 M KOH aqueous solution and then filtering, followed by further dilution with 0.10 M KOH solution.

The Au-electrode surface roughness was estimated from values of the transition time in the galvanostatic oxidation process of 3.00 × 10⁻³ mol l⁻¹ potassium ferricyanide in 0.5 mol l⁻¹ Na₂SO₄ aqueous solution, using a diffusion coefficient of 6.5 × 10⁻⁹ cm² s⁻¹ for ferricyanide [23]. A roughness factor of 1.26 ± 0.09 was obtained for three parallel experiments. In this work, a roughness factor of 1.26 was thus used in estimating the surface coverage of adsorbed molecules according to the Sauerbrey equation [10].

3. Results and discussion

3.1. Multi-mode experiments for the study of pyridoxol oxidation

The excitation and emission spectra for a 0.0973 mmol l⁻¹ pyridoxol + 0.10 M KOH aqueous solution are shown in Fig. 2. Pyridoxol itself exhibited excitation maxima at 250 and 318 nm, and an emission maximum at 383 nm, as shown in Fig. 2(a) and (c). During the voltammetric anodic oxidation of pyridoxol at potentials positive to ca. −0.1 V in 0.10 M aqueous KOH, new fluorescence spectra emerged with an excitation maximum at 360 nm and an emission maximum at 443 nm, as shown in Fig. 2(b), and the positive electrode potential shift in linear sweep voltammetry normally increased this emission maximum and the corresponding band, demonstrating that this band resulted from the electrogenerated fluorescence during pyridoxol oxidation. At λ_ex = 360 nm and λ_em = 443 nm, the fluorescence intensities of pyridoxol were very weak, as shown in Fig. 2(d), suggesting the possibility for selective fluorescence detection of the oxidation product without intervention from the electrolysis depletion of pyridoxol. It should be noted that the presence of the working electrode obviously increased the background excitation fluorescence for λ_em = 383 nm, as is seen from the comparison of Fig. 2(a) (with electrode) with Fig. 2(c) (without electrode); namely, in Fig. 2(a) the peak intensity at 250 nm became greater than that at 318 nm, and an excitation-fluorescence-intensity ramp was found near the emission wavelength above ~360 nm. The excitation-fluorescence-intensity ramp above ~360 nm may be ascribed to the light scattering at the electrode surface since the detection wavelength of 383 nm is close to 360 nm; however, the exact mechanism for the background excitation at short wavelength values (below ca. 280 nm) is unknown at present. After subtracting the background excitation spectrum recorded in 0.1 mol l⁻¹ aqueous KOH, the corrected excitation spectrum (the broken line in Fig. 2(a)) became very similar in shape to that recorded in a conventional 1 × 1 cm quartz cell without the Au electrode (Fig. 2(c)), suggesting that the background-excitation correction is still possible here. In addition, the fluorescence value obtained in 0.1 M aqueous KOH changed negligibly for both cases (λ_ex = 360 nm, λ_em = 443 nm and λ_ex = 318 nm, λ_em = 383 nm) during potential cycling, as given below, suggesting that the fluorescence emission background remained constant during the potential sweep and thus the presence of the background fluorescence is not of concern in the following findings and discussion.

Fig. 3 shows the simultaneous responses of current (I), fluorescence intensity of pyridoxol (FI_R) and its oxidized species (FI_O), and the resonant frequency (Δf_R) to potential cycling in 0.10 M KOH aqueous solution in the absence and presence of pyridoxol. Similar responses were also found in 0.1 M aqueous NaOH. In all cases the motional resistance (ΔR_I) responses were very small (not shown), suggesting that the observed frequency responses should result from the mass effect [20,22]. In the absence of pyridoxol (dotted lines), a positive potential sweep from −1.0 to ca. −0.2 V increased and then decreased the frequency, with a frequency maximum at ca. −0.6 V, and almost reversed frequency responses were observed during the negative sweep from ca. −0.2 to −1.0 V. It should be noted that, prior to the potential cycling shown in Fig. 3, the frequency response after conditioning the electrode potential at −1.0 V for 5 s was almost identical to that for 120 s, implying that the frequency response here should not result from the possible electrodeposition and
stripping of trace metal impurities in this medium. To identify the origin of the peak-type frequency response observed approximately at \(0.6\) V, an ac voltammetric measurement was conducted to estimate the effective interfacial capacitance near this potential. A staircase waveform with +50 mV steps was applied using a superimposed sinusoidal voltage with a frequency of 3.2 Hz and an amplitude of 5 mV, and the apparent interfacial capacitance, \(C_{\text{appa}}\), versus the electrode potential is shown in Fig. 4. \(C_{\text{appa}}\) exhibits a trough near \(0.6\) V, implying that this potential may be the potential of zero charge in this medium and thus the electrosorption of cations at more negative potentials and anions at more positive potentials may occur. The peak-type frequency response may thus be assigned to the double layer effect, which results in electro-sorption/desorption of ions [24]. The reduction current peak at ca. \(-0.2\) V may result from the reduction of soluble oxygen, and the frequency changed very little therein. Au started its oxidation at potentials positive to ca. \(0.2\) V during the positive sweep, with a frequency decrease occurring as expected. The surface Au oxide was gradually reduced to Au during the reverse potential scan at potentials negative to ca. \(0.2\) V, and simultaneously the frequency increased. In addition, the background fluorescence for \(0.10\) M KOH aqueous solution without pyridoxol changed negligibly in the whole potential region.

In the presence of pyridoxol, a new oxidation current peak was found at potentials positive to ca. \(-0.1\) V during the positive sweep, which increased proportionally and shifted to more positive potentials as the pyridoxol concentration was increased. The pyridoxol oxidation here was obviously irreversible, since no related reduction peaks were found during the subsequent reversal scan, and the FTO also increased significantly cycle by cycle. At relatively higher concentrations of pyridoxol, oxidation current peaks at ca. 343–351
0 V during the negative potential sweep were observed. This phenomenon may be understood as follows. Pyridoxol oxidation is facilitated on the bare Au surface but is suppressed significantly on the Au oxide. When the reduction of Au oxide to recover a fresh Au surface was initiated near ca. 0.1 V, the oxidation of pyridoxol molecules near the Au surface (remaining from the previous positive potential sweep and updated via diffusion) started to a significant extent again, since the 0.1 V potential was still located in the potential region for pyridoxol oxidation, and an increase in the oxidation current was thus observed. A similar phenomenon was also observed for glucose oxidation at a bare Au electrode in an alkaline medium [25]. At the potentials for pyridoxol oxidation (positive to ca. 0 V), the frequency responses were similar to those observed in the absence of pyridoxol, implying that here they were dominated mainly by the Au/Au-oxide redox reaction. Corresponding to the oxidation of pyridoxol in each cycle, the FIO increased and the FIR decreased simultaneously. Obviously, the FIO exhibited a much higher sensitivity to potential switching than the FIR.

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It is very interesting that, in the presence of pyridoxol, however, $f_0$ increased very slightly from −1.0 to ca. −0.75 V (region I) with negligible concomitant FIO changes, then both $f_0$ and FIO decreased from ca.
−0.75 to ca. 0 V (region II) during the positive sweep; they decreased during the negative potential sweep from ca. 0 to −0.4 V (region III) and then increased notably again, approximately from −0.4 to −0.75 V (region IV), then the frequency decreased very little but \( \Delta f \) became almost stable. The fluorescence changed similarly in regions II and IV when the concentration of pyridoxol was increased, however, the fluorescence increase in region III was observed at pyridoxol concentrations of 0.0973 and 2.92 mmol l\(^{-1}\), due probably to a very large pre-increase in fluorescence resulting from pyridoxol oxidation during the negative potential sweep from 0.1 to −0.1 V. The very complicated frequency and fluorescence responses observed in regions II−IV may be understood as follows. The adsorption of pyridoxol (partially plus its oxidation product near the electrode if present) led to the fluorescence and frequency decreases in region II.

Desorption of the oxidation product in region IV, which was preadsorbed onto the electrode surface in regions II, III and at the pyridoxol-oxidation potentials, increased the fluorescence and frequency; as reported previously, the electrode can quench the fluorescence of the oxidation product adsorbed on the electrode surface [26,27]. A simple calculation is made to strengthen this conclusion, and the results are given in Table 1. It is seen that here the ratio of the electrogenerated oxidation-product mass to the related \( \Delta f / (\Delta f_{\text{IO}}) \) associated with the potential region of the anodic oxidation (approximately from −0.2 to 0.3 V vs. SCE) is in acceptable accordance with the ratio of the mass of the desorbed species to corresponding \( \Delta f / (\Delta f_{\text{IO}}) \) associated with the desorption potential region (region IV, approximately from −0.4 to −0.75 V vs. SCE), with 90% confidence in the agreement by the paired Student’s t-test. Thus the calculation supports quantitatively the above-mentioned mechanism of oxidation-product desorption in region IV. The surface coverage of the oxidation product calculated from the desorption frequencies and the roughness-corrected surface area is \((2.9 \pm 0.2) \times 10^{-10} \text{ mol cm}^{-2}\), suggesting a monolayer-scale desorption of the oxidation product on the Au electrode surface with negligible correlation to the pyridoxol concentration from 0.0973 to 2.92 mmol l\(^{-1}\).

As for the fluorescence decrease observed in region III for 0.0973 mmol l\(^{-1}\) pyridoxol + 0.10 M KOH solution, since a concomitant frequency decrease, indicating an electrode mass gain was observed therein, one may presume that adsorption of the oxidation product still occurred at the fresh and active Au electrode surface, which was just recovered from Au oxide reduction, resulting in the observed \( \Delta f_{\text{IO}} \) decrease. It should be noted that obviously rising drifts of frequency were observed cycle by cycle in the presence of pyridoxol, implying a facilitated gold dissolution resulting probably from the complexation effect of surface Au with pyridoxol hydrochloride and its oxidation product.

Zhu et al reported via potential-controlled coulometry a 4e\(^{-}\) process for pyridoxol oxidation in alkaline aqueous solution at a pyrolytic graphite electrode, and the oxidation product of pyridoxol is pyridoxic acid (PA) [28]. Pineda studied the electro-oxidation of pyridoxal on a polycrystalline gold electrode in alkaline media by cyclic voltammetry [29]. Two significant oxidation peaks at −0.7 and −0.3 V vs. SCE, being characteristic of aldehyde oxidation at an Au electrode in an alkaline aqueous solution [30–32], were found in the positive scan during pyridoxal oxidation in 0.1 M aqueous NaOH, with pyridoxic acid as the oxidation product for both peaks. In this work, we did not observe an obvious oxidation peak near −0.7 V vs. SCE characteristic of pyridoxal oxidation in succeeding positive sweeps after the first cycle, whether we changed the scan rate from 10 to 500 mV/s or changed the positive potential limit from 0.6 to 0.3 V vs. SCE, demonstrating that the pyridoxal oxidation here at potentials positive to ca. 0 V produced pyridoxic acid directly in our experiment. By considering the adsorption/desorption phenomena described as above, a pyridoxol (PL) electro-oxidation mechanism under the experimental conditions is proposed as in Scheme 1.

Table 1
\( \Delta f_{\text{IO}} \) versus the mass gain of oxidation product in the solution at desorption potentials (region IV) and in the potential region of anodic oxidation\(^a\)

<table>
<thead>
<tr>
<th>Concentration/mmol l(^{-1})</th>
<th>Desorption potentials (region IV)</th>
<th>Potential region of anodic oxidation (=0.2−0.3 V vs. SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \Delta f / \text{Hz} )</td>
<td>( \Delta m / \text{pg} )</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>0.0973</td>
<td>8.16</td>
<td>16.9</td>
</tr>
<tr>
<td>0.973</td>
<td>8.97</td>
<td>18.6</td>
</tr>
<tr>
<td>2.92</td>
<td>9.60</td>
<td>19.9</td>
</tr>
</tbody>
</table>

\(^a\) \( \Delta m / f_{\text{IO}} \) was calculated from the Sauerbrey equation [10], \( \Delta m / f_{\text{IO}} \) was calculated from the Faraday law using the charge consumed in the potential region of anodic oxidation (background corrected), an \( n \) (number of electrons transferred) value of 4 and the molar mass of the anionic form of pyridoxic acid (181.2), and “−” indicates that the background-corrected charge consumed therein cannot be estimated (negative values) at a pyridoxol concentration of 0.0973 mmol l\(^{-1}\). Data are given for the first potential cycle.
3.2. Analytical application

The fluorescence-intensity change during pyridoxol oxidation in the first positive scan segment, ΔFI, as defined in Fig. 3, was proportional to the concentration of pyridoxol. Therefore, a new and sensitive method is presented for assay of pyridoxol by taking ΔFI as an analytical signal.

The influence of KOH concentration was examined by comparing the oxidation peak current and ΔFI of 0.0973 mmol l⁻¹ pyridoxol at KOH-concentration levels of 1.0, 0.20, 0.10, 0.050, and 0.010 M. The highest peak current and maximum ΔFI value were found in 0.10 M KOH. Therefore, 0.10 M KOH was selected for the determination of pyridoxol in this work.

Peak current and ΔFI values were recorded simultaneously at various pyridoxol concentrations. Because the F-4500 fluorescence spectrophotometer cannot record fluorescence intensity values greater than 10,000, the fluorescence signals for the higher and lower pyridoxol concentrations were recorded with different excitation/emission slit-width values, as given in the caption of Fig. 5. Linear calibration plots and related regression equations of the anodic peak current and ΔFI versus pyridoxol concentration were obtained, as shown in Fig. 5. Based on the 3σ rule, where σ is the fluorescence noise level, the detection limit of the present method is ~0.5 μmol l⁻¹. Obviously, the ΔFI-based analysis provides a higher sensitivity than the voltammetric assay, though the latter yields a wider linearity against pyridoxol concentration.

ΔFI deviated from linearity at pyridoxol concentrations above ~2 mmol l⁻¹, as a result of the so-called inner-filter effect [33]. It should be noted that a decrease of the potential scan rate increased the ΔFI value and thus improved the detection sensitivity; however, the detection time was also increased. Therefore, we selected a scan rate of 50 mV/s for assay of pyridoxol.

To examine the selectivity of the proposed method, we studied the effects of various substances on the determination of 0.0973 mmol l⁻¹ pyridoxol. A relative error of 5% was considered tolerable. K⁺, Na⁺, SO₄²⁻, NO₃⁻
and glucose did not interfere even at a 150-fold excess. Vitamin C and tyrosine started interfering at a 30-fold excess, and vitamin B1, B2 and Fe(CN) 3/4 interfered seriously even at a 2-fold concentration, due to their significant UV–Vis absorption either at the fluorescent excitation wavelength or at the emission wavelength of interest. Although those compounds having fluorescence overlapping and/or UV–Vis co-absorption interfered in the assay of pyridoxol, the present method still possesses a better selectivity than the voltammetric assay of pyridoxol, since those compounds with notable electrochemical activities but without significant spectral effects, including glucose and vitamin C, did not interfere significantly.

The pyridoxol content in several tablets was determined via the voltammetrically electrogenerated fluorescence method for testing its applicability to practical samples. Five parallel measurements were made. The results were 9.77, 9.90, 9.82, 9.71, 9.94 mg/tablet, and the average was 9.83 mg/tablet with a 1% RSD value, in accordance with the reference value labelled on the drug bottom, 10 mg/tablet. Recovery experiments for each sample gave recovery values between 98% and 103%, demonstrating the feasibility of this method for qualification of pyridoxol as a drug.

4. Conclusion

A simultaneous EQCM-fluorescence measurement setup has been developed for the study of pyridoxol oxidation on an Au electrode in KOH media. The oxidation of pyridoxol was irreversible and produced strong electrogenerated fluorescence spectra with an emission maximum at 443 nm and an excitation maximum at 360 nm. The simultaneous EQCM-fluorescence measurements have provided direct evidence for the adsorption and desorption of the oxidized product of pyridoxol at negative potentials. This novel and informative multi-mode measurement method is highly recommended for electrochemical studies of many other fluorescence-active compounds. In addition, the voltammetrically electrogenerated fluorescence has been proposed as a new protocol for a sensitive assay of pyridoxol with satisfactory results. It is also expected that the electrogenerated fluorescence method can be improved for use in flow injection analysis, or for in- or post-column detection in liquid chromatography and capillary electrophoresis.

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