ORIGINAL PAPER

Electrochemical, microscopic, and EQCM studies of cathodic electrodeposition of ZnO/FAD and anodic polymerization of FAD films modified electrodes and their electrocatalytic properties

S. Ashok Kumar · Shen-Ming Chen

Abstract Two kinds of chemically modified electrodes were prepared. In the first type of electrodes, zinc oxide (ZnO) and flavin adenine dinucleotide (FAD) molecules were deposited onto the glassy carbon-, gold-, and SnO$_2$-coated glass electrodes by using cyclic voltammetry from the bath solution containing aqueous 0.1 M zinc nitrate, 0.1 M sodium nitrate, and 1×10^{-4} M FAD. It was called as ZnO/FAD modified electrodes. The second type of modified electrode was prepared by the electropolymerization method. Electrochemical polymerization of FAD was carried out from the acidic solution containing 1×10^{-4} M FAD monomers onto electrode surfaces. This poly(FAD)-modified electrode yields a new redox couple in addition to the monomers redox couple. The influence of the concentrations, pH, and electrocatalytic properties of the ZnO/FAD- and poly(FAD)-modified electrodes are investigated by means of the in situ technique electrochemical quartz–crystal microgravimetry (EQCM) combined with cyclic voltammetry and the ex situ technique scanning electron microscopy. From these studies, it appears that the cathodic deposition of ZnO/FAD-modified electrodes gives only one redox couple, and the anodically polymerized FAD film-modified electrodes gives two reversible redox couples. The pH dependence of the redox responses were investigated and the kinetics of electron transfer was evaluated. In addition, the EQCM technique was employed to follow the deposition process of both kinds of modified electrodes in real time as well as the characteristics of the charge transfer associated with the surface-confined redox-active couples. The electrocatalytic activity of the poly(FAD)-modified electrode towards the reduction of hydrogen peroxide and the oxidation of dopamine and ascorbic acid was explored. The important electrocatalytic properties of poly(FAD)-modified electrode were observed for simultaneous separation of dopamine and ascorbic acid in neutral solution. This poly(FAD)-modified electrode has several advantages than the previously reported FAD-modified electrodes.

Introduction

Flavin is a tricyclic heteronuclear organic ring whose biochemical source is the vitamin riboflavin. The flavin moiety is often conjugated with an adenosine diphosphate to form flavin adenine dinucleotide (FAD; Scheme 1) and, in other circumstances, is found as flavin mononucleotide (FMN), a phosphorylated form of riboflavin. The flavin-containing adenine group is capable of undergoing oxidation–reduction reactions and can accept either one electron in a two-step process or can accept two electrons at once. In the form of FADH$_2$, it is one of the cofactors that can transfer electrons to the electron transfer chain. FMN is a prosthetic group found in NADH dehydrogenase and FAD is a cofactor in the enzymes D-amino acid oxidase, glucose oxidase, and xanthine oxidase. Trace amounts of FAD play an important role in physiological processes, for example, metabolism and normal growth [1, 2]. Knowledge of the behavior of FAD near an electrode surface is necessary for the correct interpretation of the electrochemistry of FAD.

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Scheme 1 Structural formula of
flavin adenine dinucleotide
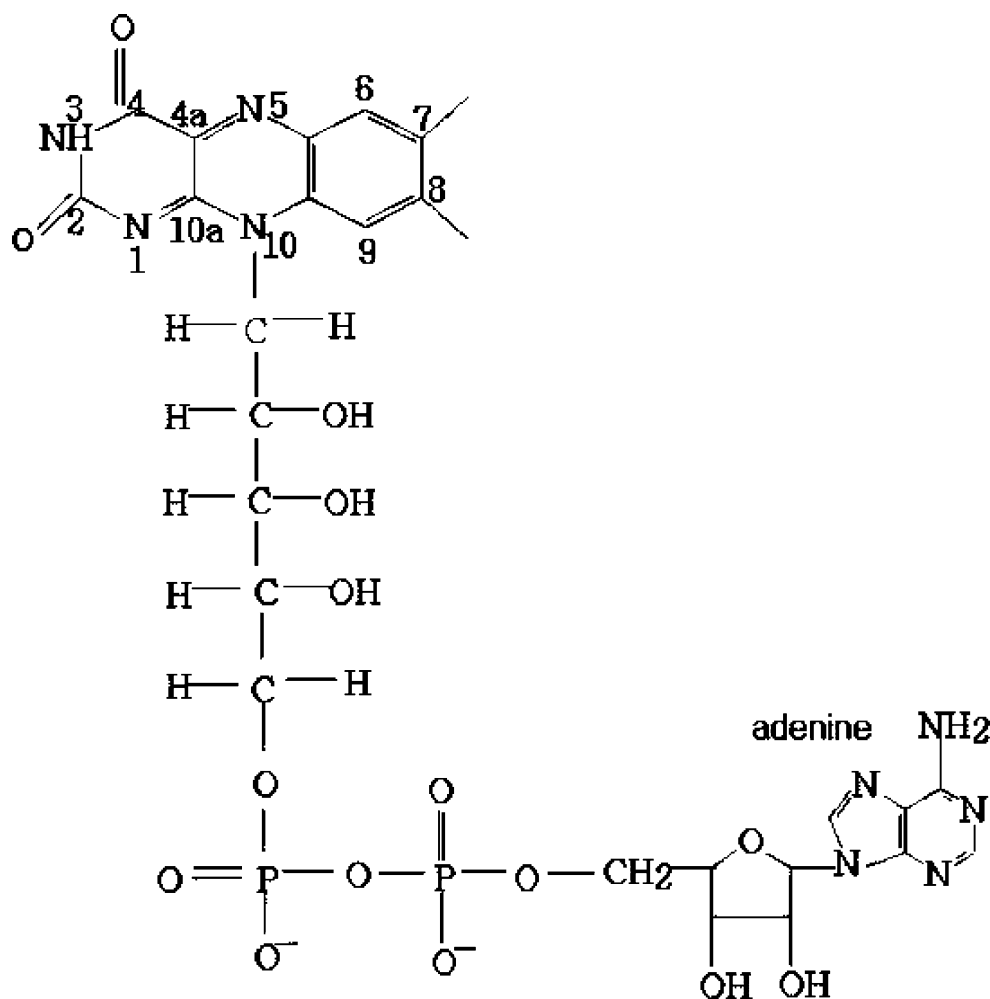
FAD has been studied quite extensively with several electrochemical techniques and several electrode materials [3–8]. Gorton and Johansson [6] found that strong adsorption of FAD did not occur at the noble metal electrodes and glassy carbon electrodes.

Nanostructured ZnO has novel applications in optoelectronics, the development of sensors and transducers, and in biomedical science, because it is biosafe. ZnO, a material that has diverse structures, has configurations that are much richer than any known nanomaterials, including carbon nanotubes. Using a solid-state thermal sublimation process and controlling growth kinetics, local growth temperature, and the chemical composition of the source materials, a wide range of ZnO nanohelixes, nanobows, nanopropellers, nanowires, and nanocages have been synthesized [9]. ZnO films are composed of nanosized metal oxide particles and have been intensively investigated in recent years for use in self-assembly dye/ZnO thin films [10–13] optoelectronic devices [14–16], and as inorganic acceptor dye-sensitized solar cells [17–19]. The zinc oxide almost exhibits n-type conductivity, with the electron in its valance band as charge carriers. Despite years of investigations, the conductivity of

ZnO is still in controversy. A recent first report reveals that none of the native defects reveal any characteristics of high-concentration shallow donors [20]. In zinc oxide, zinc is acting as a deep acceptor and oxygen in acting as deep donor. Recently our research group reported the film formation of zinc oxide/FAD-modified electrode and its interaction with hemoglobin for sensor applications [21]. However, we are still interested to know the mechanistic aspects, surface morphology, and its electrocatalytic properties. Furthermore, in this present study, we prepared the poly(FAD)-modified electrode successfully. The previous reports concerning polymerization of flavins are reported for NADH oxidation by Karyakin et al. [22], and it was described as an electropolymerization reaction leading to a polyazine with high electrocatalytic activity towards oxidation of NADH [23]. In the meantime, Alvarez et al. [24] reported a new electrocatalyst for NADH oxidation from the oxidation of FAD at highly basic condition. They also found out that the electrocatalytic activity described by Karayakin et al. was only due to the surface quinone group generation at highly positive potential (2 V) and the catalytic activity described for poly(FAD) was not due to

the polymerization. These interesting results encouraged us to study about the polymerization of flavins. In this paper, we report the successful electrochemical preparation of poly(FAD) modified electrode and its excellent electrocatalytic properties towards dopamine (DA) and ascorbic acid (AA). We also compared the cathodically deposited ZnO/FAD film-modified electrode with poly (FAD)-modified electrode based on their electrochemical properties.

In the same way, there was recently a considerable effort devoted to the development of voltammetric methods for the determination of DA and AA in biological samples. It is generally believed that direct redox reactions of these species at the surface of usual electrodes are irreversible and therefore require high overpotential [25]. Moreover, the direct redox reaction of these species at the unmodified electrodes takes place at very similar potentials and often suffers from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. The ability for selective determination of DA and AA has been a major goal of electroanalytical research [26]. Since the basal concentrations of DA and AA in biological samples vary from species to species in an extremely wide range, from 1.0×10^{-7} to 1.0×10^{-3} M [27], both sensitivity and selectivity are of equal importance in the development of voltammetric procedures. Quinones, quinoneimines, and phenylenediimines are typical catalytic functionalities. Compounds containing these functionalities have been immobilized on the electrode surface by adsorption, film entrapment, covalent attachment, or electropolymerization. A challenging problem is that AA, uric acid (UA), and DA are oxidized at nearly the same potential with poor sensitivity at solid (unmodified) electrodes, which results in overlapped voltammetric responses making their discriminations highly difficult [28]. Recently Salimi et al. [29] reported carbon composite electrode by solgel techniques. They applied their electrode system to simultaneous separation of DA, AA, and UA. They also extensively compared their experimental results with other common carbon-based electrodes. Based on the literature survey, this is the first report for simultaneous separation of dopamine and ascorbic acid using flavin-modified electrode at physiological condition. We believe these interesting electrocatalytic properties of flavins are more valuable for sensor applications.

The preparation of polymerized FAD on glassy carbon electrode yielded a new oxidation product at controlled potential (1.4 V) in aqueous acidic medium. This polymeric film had an excellent potential activity towards an overpotential of about 50 mV for dopamine (60% of peak current higher than unmodified electrode) and 280 mV for ascorbic acid oxidation. The electrocatalytic activity of poly(FAD)-modified electrodes towards the reduction of H_2O_2, oxidation of DA, and AA was also explored.

Experimental

Materials Flavin adenine dinucleotide, dopamine hydrochloride, ascorbic acid, zinc nitrate hexahydrate, and sodium nitrate were purchased from Aldrich Chemical, USA, and were used as received. Other chemicals were of analytical grade. Supporting electrolytes used for electrochemical experiments was aqueous 0.1 M sulfuric acid (H_2SO_4), 0.2 M phosphate, and 0.1 M acetate buffer solutions. The aqueous solutions were prepared by using doubly distilled deionized water, and before each experiment, the solutions were deoxygenated by purging with prepurified nitrogen gas.

Equipment Electrochemical measurements were made by means of cyclic voltammetry using a West Lafayette Model CV-50W bioanalytical system and a CH Instruments Model-400A Time-Resolved Electrochemical Quartz Crystal Microbalance. Cyclic voltammetry (CV) was performed using a three-electrode cell with a BAS glassy carbon electrode as the working electrode and a platinum wire as the counter electrode. The glassy carbon electrode was polished using 0.05 μm alumina on Buehler felt pads and then ultrasonicated for 1 min. All the cell potentials were measured with respect to a Ag/AgCl [KCl (sat)] reference electrode. The working electrode for the electrochemical quartz–crystal microgravimetry (EQCM) measurements was an 8-MHz, AT-cut quartz crystal with gold electrodes. The diameter of the quartz crystal was 13.7 mm and that of the gold electrode was 5 mm. HITACHI Model S-3000H scanning electron microscope (SEM) was used for surface image measurements.

Results and discussion

The electrochemical preparation of the ZnO/FAD film-modified electrodes

The ZnO/FAD films were deposited onto the surface of a solid electrode to form an electroactive film in aqueous solutions containing 0.1 M $Zn(NO_3)_2$, 0.1 M $NaNO_3$, and 1×10^{-4} M FAD monomers (pH 1.5). Figure 1 shows the cathodic deposition of zinc oxide films with FAD onto a glassy carbon (GC) electrode by cycling the potential between 0.2 and −0.9 V for ten cycles; after that, the modified electrode was thoroughly washed with background electrolyte solution and then transferred into
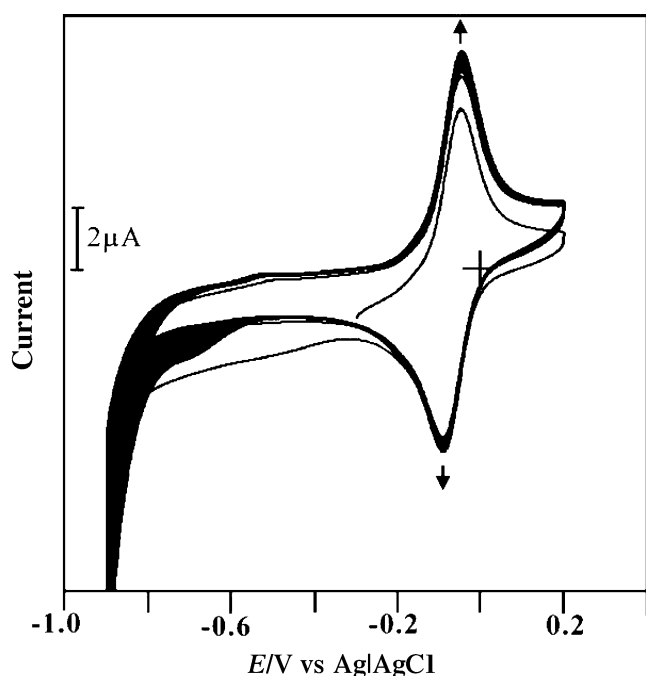
Fig. 1 Repeated cyclic voltammograms of a glassy carbon electrode in the solution containing 0.1 M $Zn(NO_3)_2$, 0.1 M $NaNO_3$, and 1×10^{-4} M FAD. Scan rate=0.1 V/s

phosphate buffer solution. The ZnO/FAD-modified electrode exhibited a single redox couple with a formal potential at −0.10 V (vs Ag|AgCl). The cyclic voltammograms of the electrochemical formation of a ZnO/FAD film onto a glassy carbon electrode were characterized by the FAD redox couple in the scanning potential region between 0.2 and −0.9 V. The continuous increase of anodic and cathodic peak currents of the FAD redox couple was indicated in the surface deposition of FAD molecules. Generally, FAD did not adsorb onto GC and noble metal electrode surfaces easily [6]. We electrochemically deposited the FAD molecules onto the electrode surface along with ZnO from the bath solution. The doping of FAD occurs due to the interaction between the phosphate group of FAD and the ZnO conduction band [21].

Electrochemical preparation of the poly(FAD) film-modified electrodes (pFADGC)

Electrochemical polymerization of FAD was carried out from the solution containing 1×10^{-4} M FAD monomer in aqueous H_2SO_4 solution. The potential was cycled between −0.5 and 1.4 V. The first cycle was started at a potential of 0.7 V towards a negative scan. The monomer reduction of FAD would occur at a potential of −0.09 V. In the second segment, the monomer oxidation peak of FAD existed at −0.05 V. During the third segment the new cathodic peak appeared at 0.25 V; the corresponding anodic peak appeared

at 0.41 V in addition to the FAD monomer peak in the fourth segment. This new set of reversible peak appeared only after the oxidation of monomer molecules at above 0.73 V. As seen in Fig. 2, upon increasing the number of cycles, the redox peak current corresponding to poly(FAD) were found to be increased obviously, as well as the peak currents corresponding to FAD adsorption. This behavior may be due to the covering of an electrode surface with poly(FAD) after the first cycle. Hence, the formation of polymerization of FAD occurred on the surface of the electrodes.

The electropolymerization of flavins occurs due to their oxidation at high anodic potentials, whereas the growth of polyazines in cyclic voltammetric regime requires the anodic switching potentials of 1.0–1.2 V [23]. Moreover, a new set of peaks, usually attributed to polymer-type redox activity, appears only upon cycling about at a potential of 0.7 V. We fixed the maximum potential for FAD oxidation at 1.4 V because this anodic potential was enough for flavin molecule oxidation. At a more positive potential, FAD molecules undergo irreversible oxidation. The previous report concerning FAD polymerization carried out in highly positive potential so there is more possibility for generation of surface quinone molecules [24]. Hence, to avoid these things in our work, we studied the effect of anodic potential on the polymerization of FAD. From these observations, we found the optimum potential window was −0.5 to 1.4 V.

It is also seen in Fig. 2 that, upon cycling, the redox activity peculiar to the FAD monomer with formal potential of (approximately) −0.07 V is increased. Electropolymerization of the known conductive polymers, like polypyrrole and polyaniline made in cyclic voltammetric conditions, is
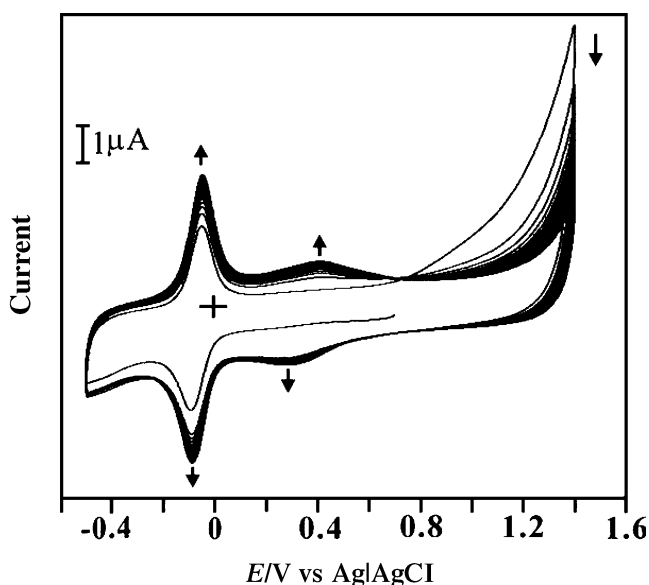

Fig. 2 Repeated cyclic voltammograms of a glassy carbon electrode in the solution containing 1×10^{-4} M FAD, pH=1.5 sulfuric acid solution. Scan rate=0.1 V/s

noticed as an appearance of polymer-type electroactivity. A new redox activity compared to the corresponding monomer was also found for electropolymerized azines. Moreover, as mentioned, adsorbed FAD does not show any additional redox peaks [30, 31]. Hence, in the course of controlled anodic oxidation of FAD, an electroactive polymer is formed. Cyclic voltammograms in riboflavin or FMN-containing solutions have shown rather similar behavior to that displayed in Fig. 2 for FAD. For all flavins, the growth rate is higher in acidic solutions than in neutral and basic media [23].

This poly(FAD) film is electroactive because each monomer unit retained its electroactive heterocyclic nitrogen atom [32]. Moreover, as polymerization occurs, more pendent amine groups become nitrogen bridges and these bridges become electroactive similar to those in polyaniline and poly(thionine) films [33]. As the potential scan was continued, the electrode was covered with a thin film. Electroactivity of the resulting modified electrodes in the supporting electrolyte solution is shown in case of FAD-modified GC (pFADGC; Fig. 3b). It is shown that monomer-type redox activity is still highly recognizable in cyclic voltammogram as a cathodic set of peaks with the formal potential of approximately −0.06 V. Peak separation of 40 mV indicates high rate of electron transfer.

EQCM study of ZnO/FAD film deposition and in situ mass measurements

EQCM is a powerful instrument that is capable of detecting very small mass changes on the gold electrode's surface that accompanies the electrochemical process occurring on the electrode surface. EQCM combined with cyclic voltammetry was used to study the in situ growth of the ZnO/FAD films formation onto gold-coated quartz crystal from the solution of 0.1 M $Zn(NO_3)_2$, 0.1 M $NaNO_3$, and 1×10^{-4} M FAD (pH 5.5). Figure 4a explains the consecutive cyclic voltammogram of a ZnO/FAD film deposition onto the gold electrode. Figure 4b demonstrates the corresponding frequency changes in the EQCM recorded during the first ten cycles of the consecutive cyclic voltammetry. The increase in the voltammetric peak current observed in Fig. 4a and the decrease in frequency (or increase in mass) noticed in Fig. 4b were found consistent with the growth of a ZnO/FAD film on the gold electrode. These results confirmed that the deposition potential of the ZnO/FAD formation occurred between −0.6 and −0.9 V (vs Ag|AgCl). In the EQCM experiments, An AT cut quartz crystal (8 MHz) overlaid with an Au film was used as the working electrode, and the mass sensitivity of the crystal is 1.4 ng cm^{-2} Hz^{-1}, which was calculated by Sauerbrey's equation [34, 35].

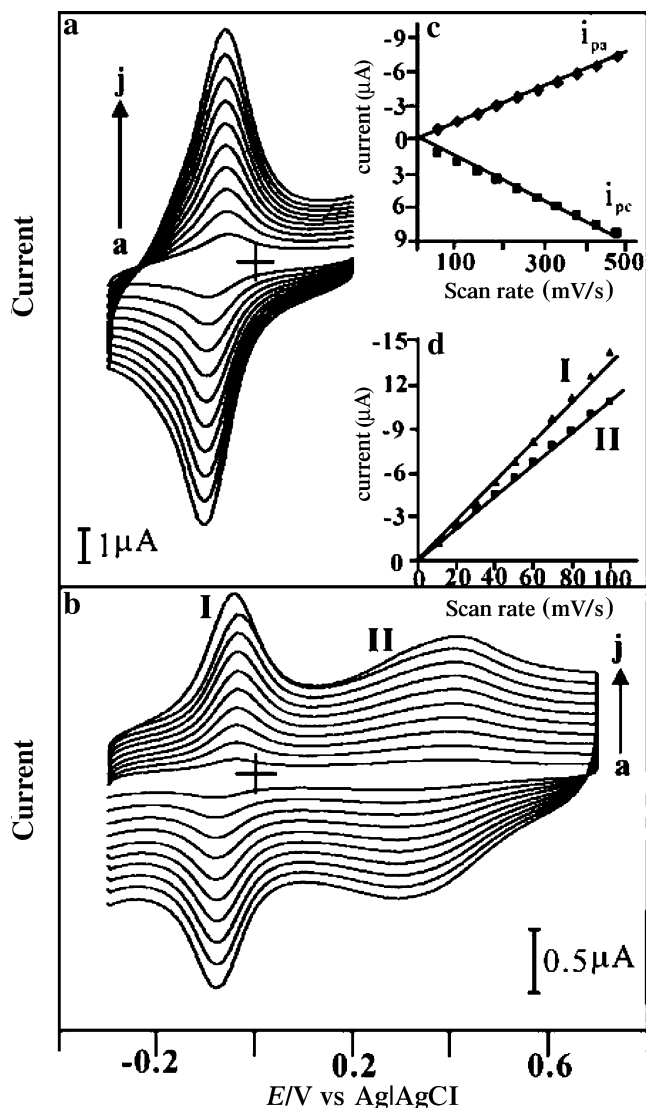$$\Delta F = -2 \Delta m f_o^2 \Big/ A \sqrt{\rho_q \mu_q} \qquad (1)$$

Fig. 3 **a** Cyclic voltammograms of a FAD/ZnO modified electrode transferred to pH=1.5 aqueous sulfuric acid solution for various scan rates. *a* 0.05, *b* 0.1, *c* 0.15, *d* 0.2, *e* 0.25, *f* 0.3, *g* 0.35, *h* 0.4, *i* 0.45, and *j* 0.5 V/s. **b** Cyclic voltammograms of a poly(FAD)-modified electrode transferred to pH=1.5 solution for various scan rates. *a* 0.01, *b* 0.02, *c* 0.03, *d* 0.04, *e* 0.05, *f* 0.06, *g* 0.07, *h* 0.08, *i* 0.09, *j* 0.1 V/s. The *inset* of **c** and **d** shows a plot of the linear increase of Ip vs scan rate

where ΔF (in Hertz) is the measured frequency shift, f_o (in megahertz) is the fundamental frequency of the quartz crystal (8 MHz), Δm (in grams) is the mass change on the electrode, A (in square centimeters) is the piezoelectricity area (0.196 cm^2), p_q is the density of quartz (2.648 g/cm^3), and μ_q is the shear modulus (2.947×10^{11} dyn cm^{-2} for AT-cut quartz). In this formula, the change in the oscillator frequency (Δf) is equal to the minus change in interfacial mass (Δm) per unit area (A) times a constant. Thus, the frequency decreases with the increasing mass.

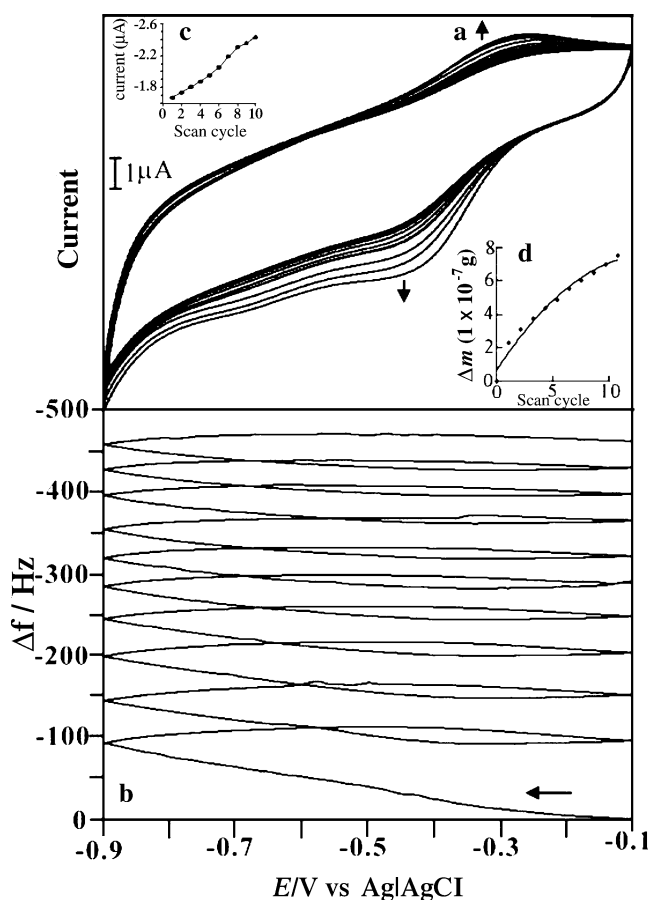When ZnO/FAD is deposited onto the electrode surface, the electrode mass increases and Δf value becomes

Fig. 4 a Repeated cyclic voltammograms of a gold electrode modified with ZnO/FAD film synthesized from 1×10^{-4} M FAD in an aqueous 0.1 M $Zn(NO_3)_2$ and 0.1 M $NaNO_3$ solution, pH 5.5, scan rate=0.02 V/s. **b** The change in EQCM frequency, recorded concurrent with the consecutive cyclic voltammograms of **a**. The *inset* shows plots of **c** the total peak current change of FAD vs scan cycle and **d** the total mass change vs scan cycle

negative; from the experimental result, the total mass of about 643.64 ng of ZnO/FAD film was deposited onto the gold electrode surface after the ten continuous cyclic voltammetric scans (Fig. 4b). After 25 continuous cycles of CVs, the gold electrode was cleaned with doubly distilled water, and then transferred into 0.1 mol^{-1} H_2SO_4 solution for CV. One reversible redox couples of monomer FAD was observed. This result was declared as our modified electrode had good stability in acidic aqueous solution.

EQCM study of ZnO film deposition and in situ mass measurements

In the same manner, EQCM and cyclic voltammetry were used to study the in situ growth of the ZnO film formation onto the gold electrode surface. Figure 5a explains the consecutive cyclic voltammogram of a ZnO film formation onto the gold electrode surface from the pH 5.5 aqueous solution containing 0.1 M $Zn(NO_3)_2$ and 0.1 M $NaNO_3$.

Figure 5b demonstrates the change in the EQCM frequency recorded during the first ten cycles of the consecutive cyclic voltammetry. The decrease in the cathodic voltammetric peak current observed in Fig. 5a and the decrease in frequency (or increase in mass) noticed in Fig. 5b were found consistent with the growth of a ZnO film onto the gold electrode surface. These results also showed that the deposition of ZnO occurred between −0.6 and −0.9 V (vs Ag|AgCl). The EQCM results again showed an obvious frequency change when the cyclic voltammetry was found more negative than −0.6 V (vs Ag|AgCl). The electro-precipitation of the ZnO thin films from the nitrate baths and the electroreduction of nitrate to nitrite ions generated OH^- ions at the cathode [10, 11]. The Zn^{2+} ions precipitated with the OH^- anions and then spontaneously dehydrated into ZnO. The overall reaction was

$$Zn^{2+} + NO_3^- + 2e^- \rightarrow ZnO + NO_2^- \qquad (2)$$
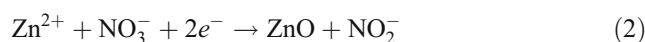
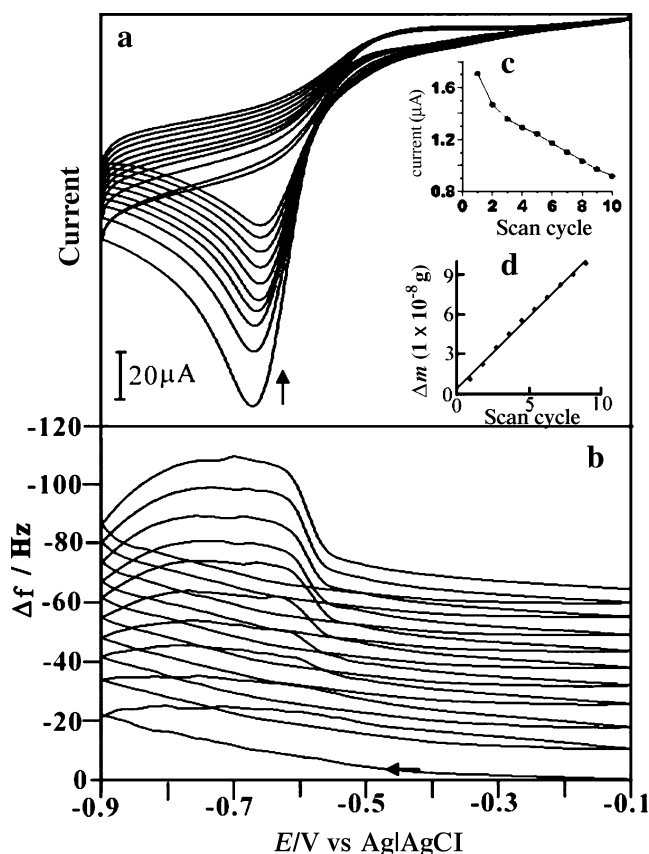In cyclic voltammogram, the cathodic peak current of nitrate reduction was decreased and the corresponding

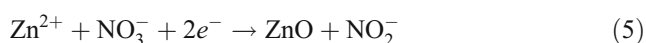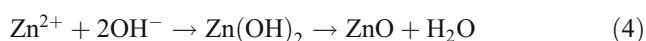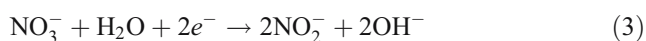**Fig. 5 a** Repeated cyclic voltammograms of a gold electrode modified with ZnO film synthesized from aqueous 0.1 M $Zn(NO_3)_2$ and 0.1 M $NaNO_3$, pH 5.5, scan rate=0.02 V/s. **b** The change in EQCM frequency, recorded concurrent with the consecutive cyclic voltammograms of **a**. The *inset* shows plots of **c** the total peak current changes of ZnO formation vs scan cycle and **d** the total mass change vs scan cycle

frequency change was observed between potential ranges of −0.5 to −0.9 V. The mass change was increased in first segment; in second segment, the mass change again decreased between −0.5 and −0.9 V. These characteristic frequency change was observed in each cycle due to the formation of zinc oxide film after the removal of water from zinc hydroxide. The reaction mechanism is described as below. The electroprecipitation of ZnO thin films from nitrate baths, however, was followed by the following reactions [10]:

$$NO_3^- + H_2O + 2e^- \rightarrow 2NO_2^- + 2OH^- \tag{3}$$

$$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2 \rightarrow ZnO + H_2O \tag{4}$$

$$Zn^{2+} + NO_3^- + 2e^- \rightarrow ZnO + NO_2^- \tag{5}$$

From these measurements, the formation of the ZnO mechanism was confirmed by EQCM and furthermore, the experimental results showed that the total mass of about 91.89 ng of ZnO film was deposited onto the gold electrode surface after the ten continuous cyclic voltammetric scans (Fig. 5b). We added a 1×10^{-4} concentration of FAD into the bath solution containing 0.1 M $Zn(NO_3)_2$ and 0.1 M $NaNO_3$ (pH 5.5), then the EQCM measurements were repeated in the same potential range. The ZnO/FAD film formation was different from the ZnO formation; it was clearly shown that the FAD molecules disturbed the ZnO formation. Yoshida and Minoura [13] observed that the chemistry at the solid/solution interface is the determinant factor; adsorption of dye molecules to the growing surface of ZnO has a significant impact on crystal growth. When water-soluble dyes such as metal complexes of 2,9,16,23-tetrasulfophthalocyanines [TSPcMs; M=Zn^{II} (TSPcZn), Al^{III}[OH] (TSPcAl) or Si^{IV}[OH]$_2$ (TSPcSi)] [36] and organic dyes such as tetrabromophenol blue or eosin Y [37] were added to the $Zn(NO_3)_2$ bath at low concentration (typically 50 mM), colored ZnO thin films with totally different surface morphologies and crystallographic structures from the pure ZnO film were obtained. In many cases, surface adsorption of dye molecules hinders crystal growth, leading to automatic formation of porous thin films composed of nano-sized crystallites of ZnO suitable for the dye-sensitized solar cells [37–41]. In some cases, anisotropy in the dye stability of the adsorption results in a preferential growth of ZnO into specific crystallographic direction to create unique textured morphologies [39]. For the application of modified electrodes by using ZnO film, we can control the film growth on the surface by using the cyclic voltammetry measurements.

On the basis of EQCM results, the FAD molecules deposited together with ZnO formation results to porous thin film formation of nano-sized crystalline. The stability of the ZnO/FAD-modified film electrode was tested by dipping the electrode in a pH-7 phosphate solution for long periods; after 30 days no significant current was decreased. This stability of electrode, which indicates the interaction between the FAD and ZnO, is very strong. FAD is envisaged to allow efficient adsorption on the semiconductor surface and, in turn, efficiently promote electronic communication between the LUMO of the FAD and conduction band of the semiconductor ZnO [38−43]. Phosphonated dyes have recently been found effective especially for solid-state dye-sensitized solar cells using electrodeposited ZnO [37].

Electrochemical polymerization of FAD and in situ EQCM mass measurements

EQCM instrument was used to study the in situ growth of poly(FAD) film onto gold electrode surface from the acidic solution containing 1×10^{-4} M FAD monomer molecules. Figure 6a,b shows cyclic voltammograms and the corresponding changes in the frequency of the EQCM recorded during the first ten cycles of the consecutive cyclic voltammetry. The voltammetric peak current in Fig. 6a and the mass increase (or decrease in frequency) in Fig. 6b are consistent with the growth of a poly(FAD) film onto the gold electrode surface. The EQCM results showed that the deposition of the film occurred between about 0.20 and 0.6 V (vs Ag/AgCl). The inset (c and d) of Fig. 6 shows a plot of the total peak current change and changes in total mass vs scan cycles of poly(FAD) formation. Then, the major frequency change occurred at the potential range between 0.2 and 1.0 V due to the polymerization of FAD. Moreover, in cyclic voltammograms, the FAD molecules exhibited oxidation peak at −0.1 V. This phenomenon indicates that the adsorption of FAD took place at bare Au surface, which was followed by the polymerized film formation on gold electrode surface.

When FAD molecules adsorb onto the electrode surface, the electrode mass increases and Δf value becomes negative, from the experimental result the total mass about 148 ng of poly(FAD) film was deposited onto the gold electrode after the first ten cyclic voltammetric scans (Fig. 6b). After ten continuous cycles of CVs, the gold electrode was cleaned with doubly distilled water and then transferred into 0.1 M H_2SO_4 sxolution for CV. Two reversible redox couples of monomer FAD and polymer-type redox peaks were observed. This result declared that our polymerized modified electrode had good stability in aqueous acidic solution. The previous report shows that the FAD undergoes only redox reaction on gold surface, but it was not stable in blank buffer. This clearly indicates the polymerized film was formed in our case [1].
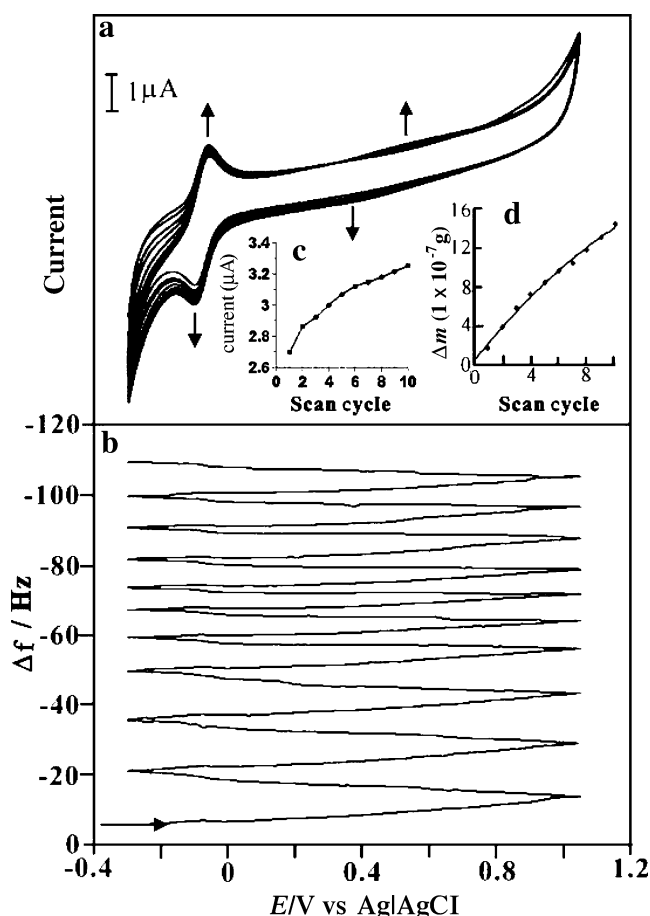
Fig. 6 a Repeated cyclic voltammograms of a gold electrode modified with poly(FAD) film from a pH=1.5 aqueous H₂SO₄ solution containing 1×10^{-4} M FAD solution. **b** The change in EQCM frequency, recorded concurrent with the consecutive cyclic voltammograms of **a**. Scan rate=0.02 V/s. **c** The change in total peak current of poly(FAD) vs scan cycle. Subpanel **d** shows plots of the change in total mass vs scan cycle

The electrochemical properties of the ZnO/FAD-modified electrodes

The electrochemically modified ZnO/FAD GC electrode was transferred into aqueous sulfuric acid solution (pH 1.5). It showed one obvious redox couple in the scanning potential ranges of −0.1 and −0.4 V (vs Ag|AgCl). Figure 3a and the inset of Fig. 3c shows that a linear increase of peak current with respect to scan rate were observed. This behavior of the ZnO/FAD films on a glassy carbon electrode suggests facile-charge transfer kinetics within the range of sweep rates and reversible electron transfer process at low scan rates. The peak current and scan rate are related as below [44, 45]:

$$Ip = n^2 F^2 \upsilon A \Gamma_o / 4RT \qquad (6)$$

where n represents the number of electrons involved in the reaction, A is the surface area (0.0707 cm²) of the GC, Γ

(in moles per square centimeter) is the surface coverage, and other symbols have their usual meanings. The cyclic voltammograms depict that in the redox couple of the ZnO/FAD film, the value of $\Delta E_p = (E_{pc} - E_{pa})$ was nonzero when the scan rate was from 10 to 500 mV/s. The value of ΔE_p is dependent on the scan rate and the pH (proton concentration), the value of formal potential ($E^{0\prime}$) also depended on the pH of the buffer solution. The reduction process of FAD consisting of two hydrogen atoms and two electron transfer process is well-known (Scheme 2). The value of ΔE_p as nonzero may be explained by the redox couples of ZnO/FAD films having some proton transfer capability and the proton transfer could be a transport hindrance on the redox process of the modified film. Proton ion diffusion controlled the electrode redox process of the FAD/ZnO film-modified electrodes. The results illustrated that the FAD reactions were electrochemically reversible in aqueous solution when using the bare glassy carbon electrode. This behavior was noticed due to the reversible electron transfer process involving the FAD along with a second diffusion process occurring alongside the electron transfer process [21, 46]. The ZnO/FAD-modified electrode was a good choice for the electrocatalytic reduction of oxygen, hemoglobin, hydrogen peroxide, and NAD⁺ [21]. In this work, we demonstrate the study of electrocatalytic properties of poly(FAD) and its electrocatalytic activity towards the separation of dopamine and ascorbic acid are described.

The electrochemical properties of poly(FAD) film-modified electrode

Figure 3b shows the cyclic voltammograms of the poly(FAD)-modified electrode obtained in pH 1.5 aqueous H₂SO₄ solutions. This indicated that the modified electrode exhibits two chemically reversible redox couples with formal potentials ($E^{0\prime}$) at about −0.06 and 0.36 V (vs Ag/AgCl) corresponding to monomer FAD molecules and poly(FAD), respectively. The peak currents of monomer FAD molecules and poly(FAD) increases linearly with scan rate up to 100 mV/s. The plot of the linear increase of peak currents vs scan rate is shown in the insets of Fig. 3d. These results show that the poly(FAD) film was both stable and electrochemically active in the aqueous buffer solution. According to the slope of the Ip–υ curve, the surface coverage (Γ) was estimated from integration of the reduction peak in the CVs according to $\Gamma = Q/nFA$, where Q is the charge involved in the reaction, n is the number of electron transferred ($n=2$), F the Faraday's constant, and A is the electrode Area (0.0707 cm²). The surface concentration (Γ) of poly(FAD) was 3.28×10^{-8} mol/cm². It was shows that the behavior of the poly(FAD) film on a glassy carbon electrode was a surface-confined redox process and a reversible electron transfer process.

Scheme 2 The redox reactions of FAD molecules
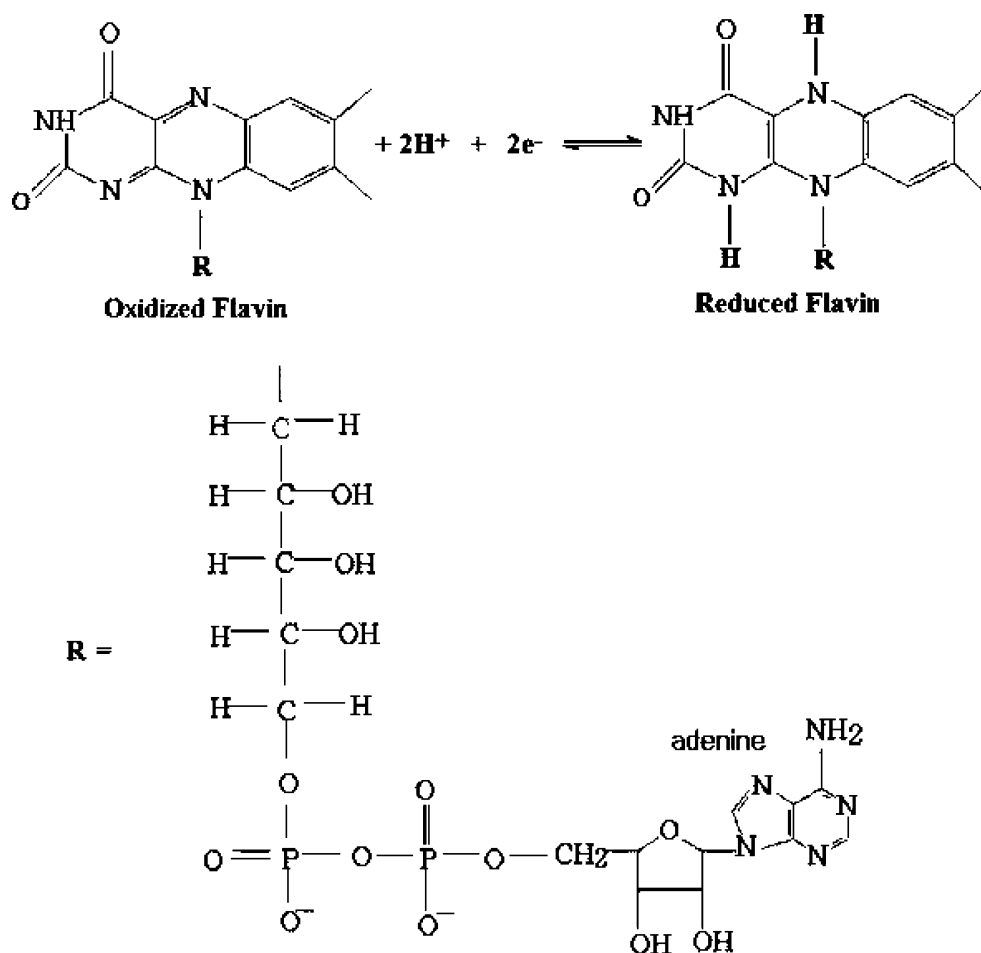
The effect of the poly(FAD) film's thickness was controlled by the number of CV cycles during the electro-polymerization and the corresponding surface coverage's was calculated by using the equation $\Gamma = Q/nFA$. It was interesting to observe that there was a rapid increase in surface coverage of poly(FAD) for up to ten cycles after polymerization. After the tenth cycles, the surface coverage slowly tended to reach the limiting value. In the present paper, the poly(FAD) film thickness was controlled by the number of cycles during cyclic voltammetry. However, the thickness of the poly(FAD) was measured using atomic force microscopy technique (not shown here) and that was found as 84±10 nm for ten cycles in the same experimental conditions used for polymerization of FAD. Hence, the suitable poly(FAD) thickness for further analysis was kept at 84±10 nm.

SEM measurements of ZnO/FAD, ZnO and Poly(FAD)-modified electrodes

To ascertain the surface morphologies of the ZnO/FAD, ZnO and poly(FAD) films were deposited onto the cleaned SnO₂ (ITO) coated glass surface. SEM images of ZnO/FAD-modified electrode (Fig. 7a) indicated that porous beadlike particles appeared, the size of the particles in the ranges between 0.2 and 2 μm. Figure 7b shows the surface morphology of pure ZnO-coated ITO electrodes. It appeared like bundles of needlelike structures, the size of the ZnO needles between 1and 4 μm. For comparison, we compared the SEM images of ZnO/FAD and pure ZnO; it was understood that the FAD molecules very much disturbed the crystallike formation of ZnO at room temperature. Figure 7c reveals that the poly(FAD) has been formed onto the ITO electrodes; furthermore, the image reveals that porous polymer film evenly distributed across the electrode surface.

Effect of solution pH on poly(FAD) modified electrodes

Figure 8 shows that the cyclic voltammograms of poly (FAD)-modified GC electrode exhibited two reversible redox couples in the pH range 1<pH<10. The values of $E^{0\prime}$ were dependent on the pH of the buffer solutions. The inset of Fig. 8 shows the formal potential of the monomer FAD molecules and poly(FAD) film vs pH of the solution; the gradients were 57.0 and −60. 4 mV/pH for FAD molecules and poly(FAD), respectively. These values are very close to that expected from calculations using the
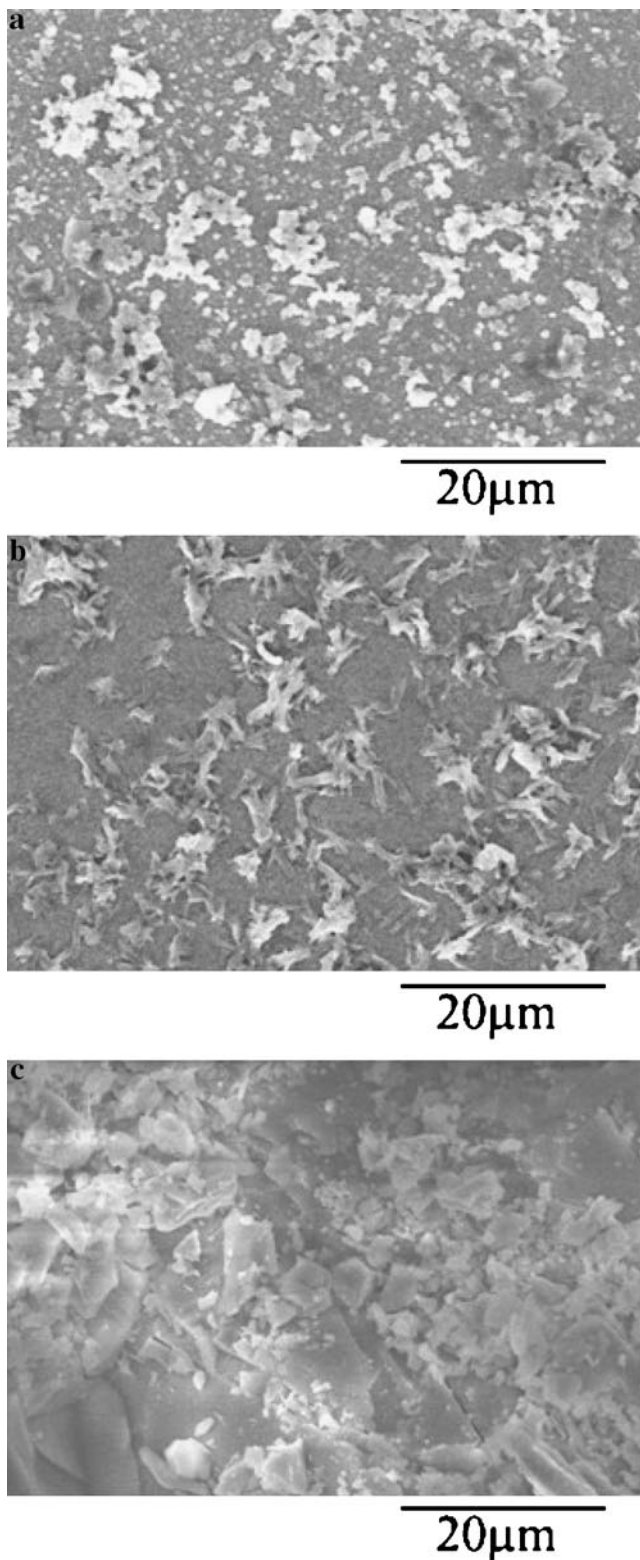
Fig. 7 a SEM images showing the surface morphology of FAD/ZnO-modified ITO electrode. **b** Only ZnO and **c** poly(FAD) modified electrodes

Nernst equation. This phenomenon indicates that the number of electrons and protons is the same, whereas two electrons and two protons are involved in the FAD redox couple. The value of $E^{0\prime}$, which depends on the pH, also shows that the redox couple of the polymeric film includes some proton transfer in the reduction and oxidation processes. The pH response was due to the proton transfer from FAD (the oxidized quinone form) to the reduced $FADH_2$ (the fully reduced hydroquinone form). We also synthesized the polymeric film at different pH conditions. The results indicated that the highly basic condition is not suitable for polymer formation; it means that the recent report for NADH oxidation using FAD oxidation product at highly basic condition is a different one [24]. Hence, acidic condition is an optimum for polymer growth (figure not shown). We also studied the effect of the anions on the polymer film formation; the result reveals that chloride ions (Cl^-) easily adsorbed onto carbon electrodes at such high anodic potentials. Furthermore, we are used SO_4^{2-}, NO_3^-, ClO_4^- as supporting electrolytes for poly(FAD) film synthesis. It was found out that there is no interference due to these anions, except chloride ions, onto the polymer film formation.
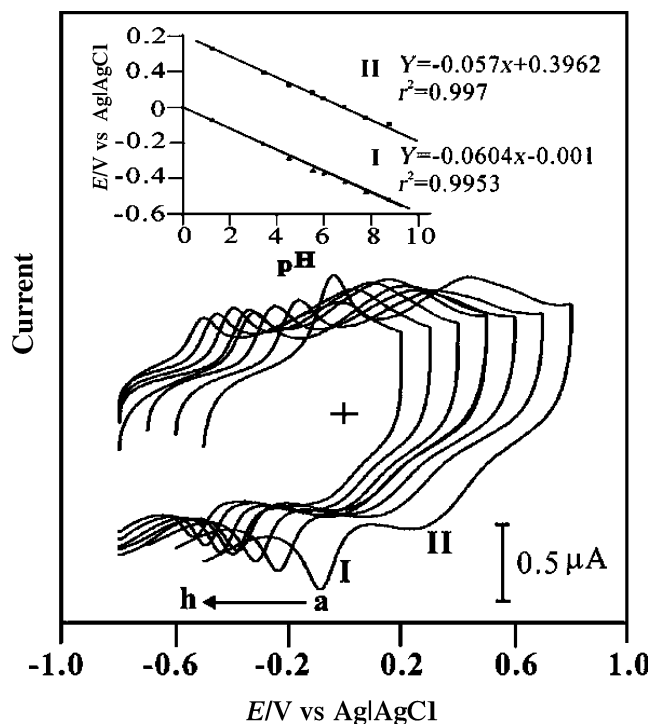

Fig. 8 Cyclic voltammograms of a glassy carbon electrode modified with a poly(FAD) film after being transferred to aqueous solutions with various pH: *a* 1.3, *b* 3.3, *c* 4.5, *d* 5.5, *e* 6.0, *f* 6.9, *g* 7.8, and *h* 8.8. The *inset* shows a plot of the formal potential vs pH, where *I* is a plot of the formal potential of FAD vs pH, and *II* is a plot of the formal potential of poly(FAD) vs pH. Scan rate=0.1 V/s

Electrocatalytic reduction of H_2O_2 at poly(FAD)-modified GC electrodes

The electrocatalytic reduction of H_2O_2 at a pFADGC-modified electrode in pH 7.0 phosphate buffer solution was studied by cyclic voltammetry. The electrochemical responses are shown in Fig. 9. The catalytic peak current Ip_{cat} (the cathodic peak potential, Ep_{cat}, at a potential of about −0.47 V) of the poly(FAD) film of $FADH_2/FAD$ redox couple was increased. This arose from the direct electrocatalytic reduction of H_2O_2 through the $FADH_2$ catalyzed by the FAD molecules. The cathodic peak current of FAD increases, and the decrease in anodic peak current for each addition indicated the mediated reduction of hydrogen peroxide at pFADGC-modified electrode.

Electrocatalytic oxidation of AA and DA at poly(FAD)-modified GC electrodes

The cyclic voltammograms obtained for a GC electrode, before and after the modification by poly(FAD) and in the absence and presence of AA, were also obtained. A 0.2-M phosphate buffer solution (pH 6) was used as supporting electrolyte. It was observed that at unmodified GC electrode, AA exhibits an anodic peak at about 400 mV (vs ref.) in forward scan, but no cathodic counterpart is observed in reverse scan, indicating a totally irreversible behavior for the electrode process. Whereas, at pFADGC-modified electrode, an enhanced anodic peak at a less positive potential of 120 mV (vs ref.) was obtained for AA, and the cathodic peak of the modifier disappeared completely in reverse scan, which is indicative of an electro-mediated process. By comparison of these peak potentials,

it can be concluded that the pFADGC-modified electrode causes a decrease in overpotential of ca. 280 mV and an increase in anodic peak current (Fig. 10). The optimum pH for electrocatalytic oxidation of AA at the modified electrode was found to be 6. The pFADGC-modified electrode also showed a potential catalytic activity towards dopamine, the modified electrode gives a highly reversible peak for dopamine (peak separation was 20 mV), and peak current was increased higher than 60% when compared to bare GCE (Fig. 11). Cyclic voltammograms obtained at pFADGC-modified electrode for solutions containing various concentrations of AA or DA exhibited the voltammograms for which the peak current were linearly proportional to the substrate concentration in solution. This behavior permits us to plot the corresponding calibration curves. On the basis of such curves, a linear dependence exists between peak currents for AA and DA concentrations ranging from 5×10^{-3} to 0.3 μM and 2.5×10^{-3} to 0.04 μM. The calculated limit of detection was 0.005 μM, with the correlation coefficient (r^2) 0.9992 and 0.9981, respectively.

Simultaneous determination of AA and DA

Another unique advantage of pFADGC-modified electrodes was the simultaneous determination of ascorbic acid and dopamine in aqueous solution. Figure 12 depicts the linear sweep voltammograms that were obtained for dopamine and ascorbic acid coexisting in pH 6 phosphate buffer solution at the pFADGC-modified electrodes. The curve a shows the cyclic voltammogram that was obtained for the solution containing 0.01 μM of AA and 5×10^{-3} μM of DA
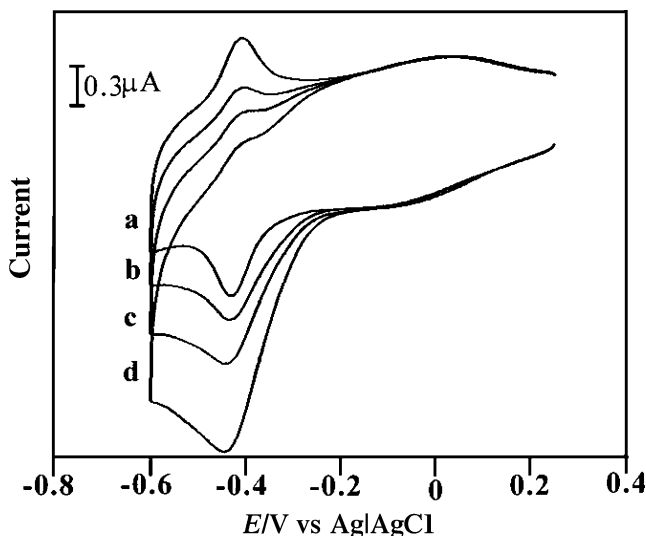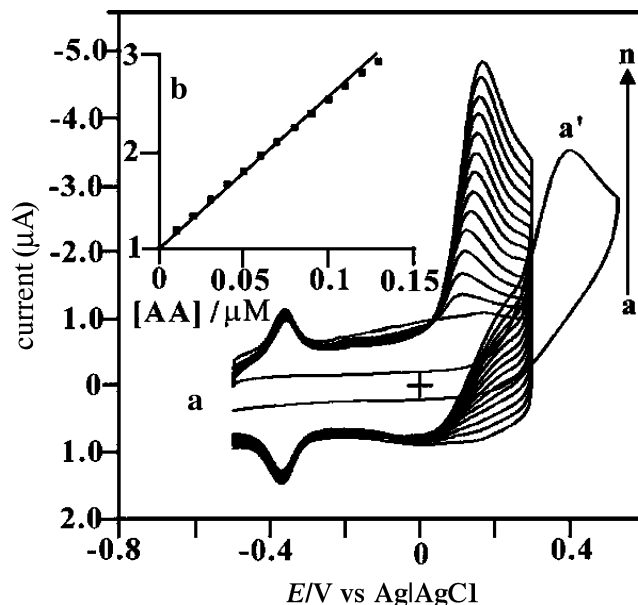

Fig. 9 Cyclic voltammograms of a poly(FAD) film adhered to a glassy carbon electrode in 10 ml of a deoxygenated phosphate (pH 7.0) buffer solution with [H_2O_2] equal to *a* 0, *b* 0.05, *c* 0.1, and *d* 0.2 μM

Fig. 10 **a** CVs of the poly(FAD) film in the aqueous phosphate buffer (pH 6.0) solution containing different concentrations of ascorbic acid of *a* 0.0, *b* 0.01, *c* 0.02, *d* 0.03, *e* 0.04, *f* 0.05, *g* 0.06, *h* 0.07, *i* 0.08, *j* 0.09, *k* 0.1, *l* 0.11, *m* 0.12, and *n* 0.13 μM. (**b**) Inset shows Ip_a vs [AA]
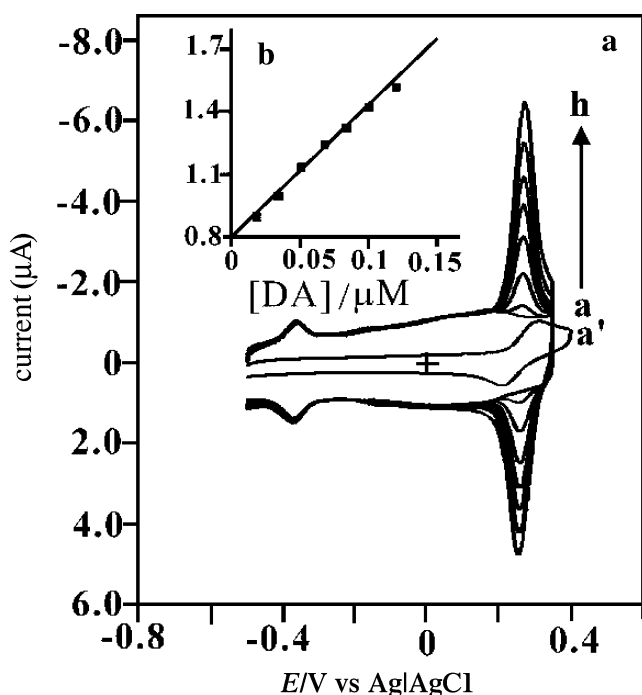
Fig. 11 **a** CVs of the poly(FAD) film in the aqueous phosphate buffer (pH 6.0) solution containing different concentrations of dopamine of *a* 0.0, *b* 2.5×10^{-3}, *c* 5×10^{-3}, *d* 7.5×10^{-3}, *e* 1×10^{-2}, *f* 1.3×10^{-2}, *g* 1.5×10^{-2}, and *h* 1.8×10^{-2}, μM. (**b**) Inset shows I_{p_a} vs [DA]

mixture in pH 6 phosphate buffer solution at the pFADGC-modified electrode. The modified surface separated the interference peak of AA from DA. Only a single broad peak was obtained at an unmodified GC electrode. These observations clearly indicated that the bare glassy carbon electrode failed to separate the voltammetric signals of AA
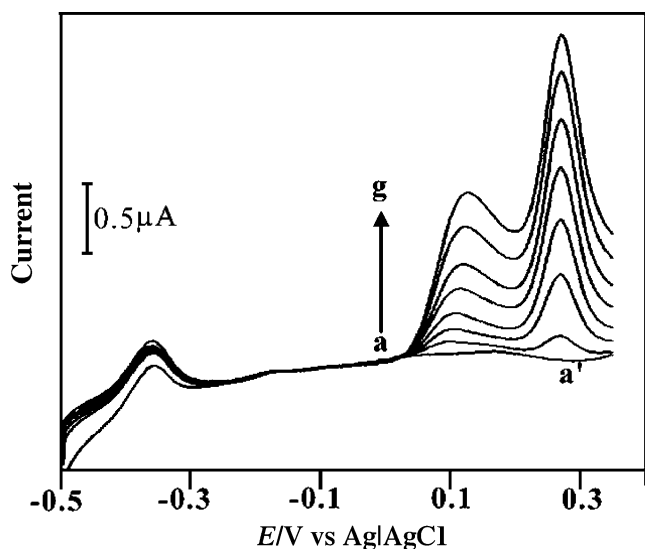

Fig. 12 **a** Linear sweep voltammograms recorded for a mixture containing *a'* 0.0 μM AA and DA *a* 0.01 AA, 0.005 DA; *b* 0.02 AA, 0.01 DA; *c* 0.03 AA, 0.015 DA; *d* 0.04 AA, 0.02 DA; *e* 0.05 AA, 0.025 DA; *f* 0.06 AA, 0.03 DA; and *g* 0.07 AA, 0.035 DA μM, respectively, in aqueous phosphate buffer (pH 6.0) solution at poly (FAD) modified GC electrodes

Table 1 The electrocatalytic properties of ZnO/FAD and poly(FAD) modified electrodes

Modification of electrode surface	Analyte	Catalytic properties	References
ZnO/FAD	O_2	Reduction	[21]
	NAD	Reduction	[45]
	H_2O_2	Reduction	[21]
Poly(FAD)	Ascorbic acid	Oxidation	tw
	Dopamine	Oxidation	tw
	NADH	Oxidation	[22]

tw This work

and DA. The fouling effect of the electrode surface with the oxidized products and the catalytic oxidation of ascorbic acid by the oxidized product of dopamine were the reasons for obtaining the single peak for ascorbic acid and dopamine mixture [47]. The curve (a–g) of Fig. 12 shows the simultaneous addition of AA and DA; the two corresponding peaks was increased for each addition at pFADGC-modified electrode. The pFADGC-modified electrode exhibited two well-defined oxidation peaks at 0.13 and 0.27 V corresponding to the oxidation of ascorbic acid and dopamine, respectively, since the oxidation peak potential of ascorbic acid was shifted to less positive potential and the oxidation peak potential of dopamine was also shifted to 50 mV less positive potential than the unmodified GC electrode. This section concludes that the modified electrode surface has a potential activity for simultaneous determination of AA and DA in real samples also.

This unique property of poly(FAD) is a new identity of flavins. This behavior will enhance its role in biosensor applications. Recently, our research group reported that the hybrid film modified electrodes by using FAD with poly (luminal) [48] for the reversible electrocatalytic properties with NAD^+ and NADH. This poly(FAD)-modified electrode is a simple method for electrode preparation; furthermore, it could be useful to study the reversible reaction of NADH oxidation and NAD^+ reduction, because it is not required more than one compound for modification. The electrocatalytic properties of ZnO/FAD- and poly(FAD)-modified electrodes for various analytes are listed in Table 1.

The better separation of the redox species such as AA and DA at the poly(FAD) film-modified electrode can be understood in the following section. The equilibrium distribution of species between an ionic polymer (p) and liquid (s) phase can be written in terms of the local relaxation, electrostatic and resolution Gibbs energies of transfer [49].

$$RT \ln K = RT \ln \left(\gamma_s / \gamma_p \right) - ZF \left(\Phi_p - \Phi_s \right) - \left(\mu_p^o - \mu_s^o \right) \tag{7}$$

where γ is the activity coefficient of the species, which is expressed as the ratio of its activity to analytical concentration; Z is its ionic charge; μ_0 is its standard chemical potential; Φ is the electric potential in the considered phase; and all other symbols have their usual meanings. Although the Donnan potential $(\Phi_p - \Phi_s)$ is positive for cationic polymers such as poly(FAD) [49], considering the electrostatic Gibbs energy of transfer for the charged species and poly(FAD) has a charged-site concentration of 1.5 M, it includes the same concentration of charge compensating counterions; thus, it can form a solution like polyelectrolyte gel in an aqueous environment [49, 50]. For such a polymer, one may assume the resolution Gibbs energy $\left(\mu_p^o - \mu_s^o\right)$ to be zero. The negatively charged AA at neutral pH seems to form a weak complex with poly(FAD). The binding of AA to the pFADGC-modified electrode results from a molecular interaction between the positive charge of poly(FAD) and AA. Kearney et al. [51] showed that the cation–π interaction can cause synthetic hosts to have novel binding selectivities and high affinities even for highly water-soluble guests. We have only limited information on such intermolecular interaction, leading to the complex formation in solution. Therefore, overpotential for AA oxidation is very much reduced due to charge-compensating effect, and better separation of AA and DA could be succeeded at the pFADGC-modified electrode with excellent selectivity and sensitivity. These interesting features of poly(FAD) film-modified electrode offer great promise for in vitro and in vivo applications.

The stability of the modified electrodes such as poly (FAD)/Au and poly(FAD)/GC was examined using cyclic voltammetric method in supporting electrolytes. The peak currents corresponding to the poly(FAD)-modified GC electrode almost remained constant for about 200 cycles. We also kept the poly(FAD)-modified GC electrode in phosphate buffer for 1 month; after a month, the peak current was decreased up to 1%. These studies indicated that two kinds of chemically modified electrodes could be prepared by using FAD molecules. These modified electrodes had good stability and, furthermore, poly(FAD)-modified electrode was successfully applied for simultaneous detection of dopamine and ascorbic acid.

Conclusions

It is possible to prepare two kinds of chemically modified electrodes by using FAD molecules depending on our need. EQCM and SEM were used to study the deposition mechanism and surface morphologies of ZnO/FAD, ZnO and poly(FAD) film formations. Cathodic deposition of FAD with ZnO yields a single reversible redox couple and an oxidative polymerization of FAD on glassy carbon; ITO and gold electrodes by employing repetitive cyclic voltammetry gives rise to a very stable redox-active polymer. The synthesis medium used for the electrochemical polymerization is a strongly aqueous acidic solution containing monomer 1×10^{-4} M FAD molecules. The influence of the concentrations, pH, and electrocatalytic properties of both kinds of films are investigated by means of in situ techniques using EQCM combined with cyclic voltammetry and ex situ technique scanning electron microscopy. The experimental results indicated that the redox processes of poly(FAD) films are confined to the surface, confirming their immobilized state. Film thickness of poly(FAD) can be easily controlled by a number of potential cycles applied in the polymerization step; the optimum thickness was found as 84 ± 10 nm. The resulting film is permeable, since ascorbic acid and dopamine exhibits potent and persistent electrocatalytic activity toward this species. These properties may be very valuable in the design of biosensors based on dehydrogenases. Our modified electrode was proven to be effective in this reaction. The structure of electropolymerized flavins is also expected to be similar to a previous model approved for polyazines [22, 23].

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