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Electrochemical study of riboflavin adsorbed on a graphite electrode

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Electrochemical redox processes of riboflavin adsorbed on a graphite electrode have been studied by cyclic voltammetry, and two separate steps for anodic oxidation of a reduced form of riboflavin have been found at a sufficiently fast potential sweep. The midpoint redox potential (–0.460 ± 0.002 V *vs.* SCE at pH 7.0) and the charge transfer rate constant (0.05 s^{-1}) have been determined.

Key words: riboflavin, redox, cyclic voltammetry, graphite electrode

INTRODUCTION

Riboflavin (vitamin B_2) plays an important role in living processes as a precursor of coenzymes FMN and FAD. The core structure of riboflavin, an isoalloxazine ring, participates in enzyme-catalysed electron transfer processes of many important metabolites. Therefore, electrochemical redox processes of riboflavin have been studied thoroughly with the use of different techniques [1, 2]. While most of the works relate to solution electrochemistry of riboflavin, it is of interest to study this species adsorbed or immobilized on electrode surface. Graphite electrode presents a suitable hydrophobic surface able to adsorb many organic redox couples, enabling to study electrochemical charge transfer processes [3, 4]. The present work has been aimed to study the redox processes of riboflavin adsorbed on a graphite electrode.

EXPERIMENTAL

Riboflavin was adsorbed from its saturated aqueous solution for 30 s on a flat circular graphite electrode 3 mm in diameter, mounted into a plastic electrode holder. Electrochemical experiments were done in 0.1 M Tris-HCl buffer solution pH 7.0, containing 0.1 M of KCl, with the use of a BAS potentiostat (Bioanalytical Systems, USA). Platinum wire and a saturated calomel electrode were used as a counter- and reference electrodes, respectively.

RESULTS AND DISCUSSION

After adsorption of riboflavin, a graphite electrode shows well defined cathodic and anodic peaks which correspond to a reversible reduction and oxidation of adsorbate (Fig. 1). The cyclic voltammograms observed do not change after a prolonged potential cycling within the potential limits used, indicating that the adsorbed layer of riboflavin does neither desorb nor degrade electrochemically in the study conditions. Integration of both cathodic and anodic peaks obtained at a relatively low potential scan rate not exceeding 50 mV/s gave nearly coinciding values of the electric charge passed from which, assuming a two-electron redox reaction to proceed, a surface coverage of ca. 7 nmol/cm^2 was calculated. This value is closely related to those obtained for other adsorbates of a similar structure, *viz.* the redox dyes Nile blue and Meldola blue [4]. Assuming the surface coverage for the adsorbate monolayer to be within the limits of 0.1 to 1.0 nmol/cm^2 depending on the roughness of electrode surface, it could be concluded that, under the conditions used, a few or a few tens of riboflavin monolayers are adsorbed on the graphite surface.

The values of cathodic peak current depend linearly neither on the scan rate as could be expected for a monolayer coverage of the electrode by electroactive species [5] nor on the square root of scan rate as could be expected for diffusion-controlled redox processes [6]. The linearisation of the data obtained for the scan rate (v) ranging from 2 to 2000 mV/s in double logarithmic coordinates, *viz.* log i_{pc} on log v, yields a linear dependence with the slope of 0.70 (with r = 0.998 for n = 10) (Fig. 2). This indicates a mixed behaviour of the system studied, i. e. an intermediate case between the diffusion-controlled redox process and the surface-bound redox reaction. Most probably the deviation from the behaviour characteristic of a surface-located process is caused by a relatively slow charge propagation (diffusion) through the layer of adsorbed riboflavin, consisting of a few or a few tens of monolayers.

For a reversible diffusion-controlled electrochemical process, the peak potential does not depend on the potential sweep rate, whereas, for an irreverble redox process, the position of the

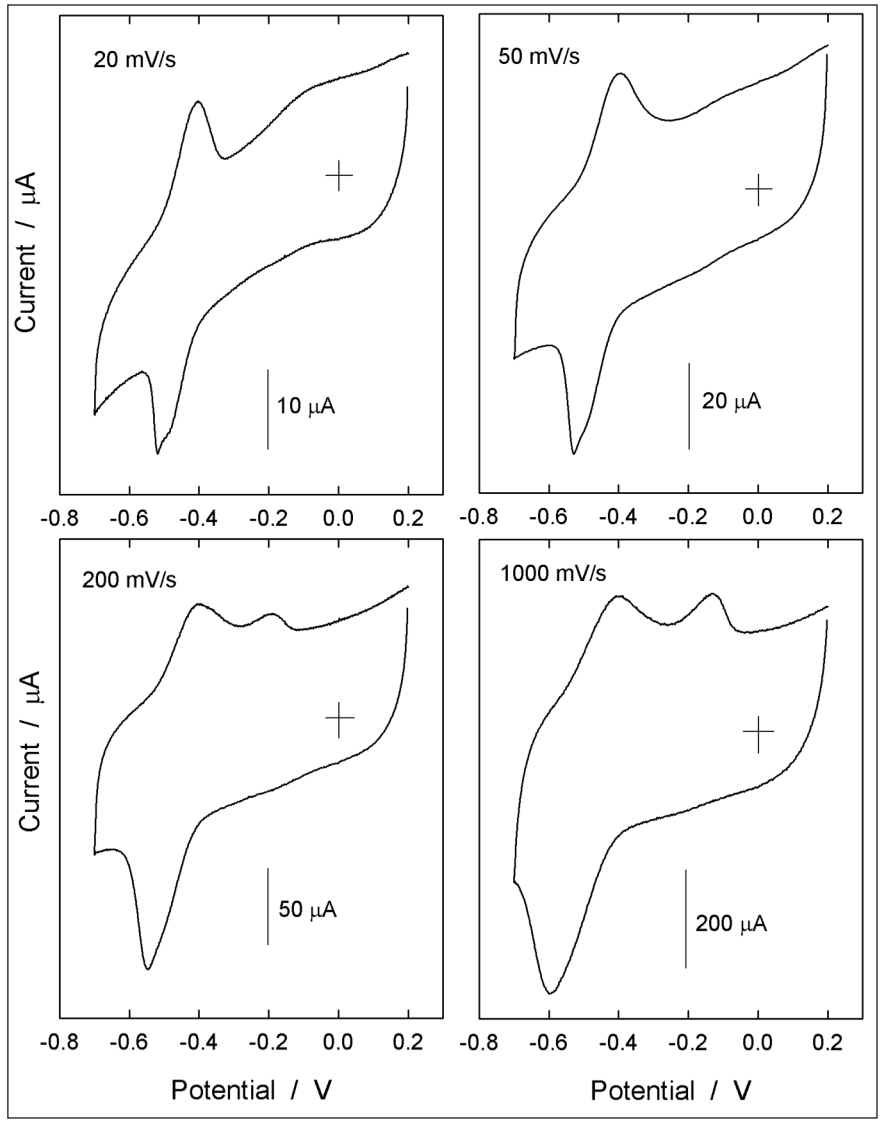

Fig. 1. Cyclic voltammograms of riboflavin adsorbed at a graphite electrode, as obtained in 0.1 M Tris-HCl buffer solution pH 7.0, containing 0.1 M of KCl, within potential scan limits of 0.2 to –0.7 V *vs.* SCE at a different scan rate ranging from 20 to 1000 mV/s (as indicated)

current peak on the potential scale is described by the following equation [7]:

$$Ep = E½ + (b/2) \bullet [1.04 - \log(b/D) - 2\log kf + \log v],$$

where $E_{½}$ is the half-wave potential, b – Tafel slope, D – diffusion coefficient, k_f – rate constant for the forward reaction, and v – potential sweep rate.

The data obtained for different scan rate are plotted in Fig. 3 in accordance with this equation. Linear relationships are obtained from the data for both anodic and cathodic (up to $v = 200$ mV/s) processes, yielding different slopes, which reflects a different grade of electrochemical reversibility. The greatest slope, and thus the least reversibility, is observed for the second anodic process that appears at a fast potential sweep. Data presented in Fig. 3 show that electrochemical reduction and oxidation of adsorbed riboflavin appears to be, at least partially, a diffusion-controlled process.

An average of midpoint potential values $E_m = (E_{pa} + E_{pc}) / 2$, obtained from five cyclic voltammograms recorded at a relatively low potential sweep rate within the limits of 2 to 50 mV/s, equals to (–0.460 ± 0.002) V *vs.* SCE, *i. e.* –0.21 V *vs.* RHE. For riboflavin

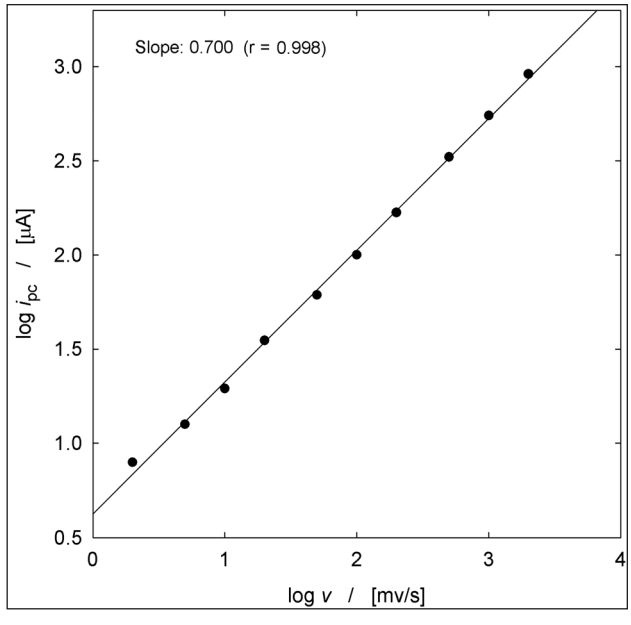

Fig. 2. Dependence of cathodic peak current on potential scan rate in double logarithmic coordinates

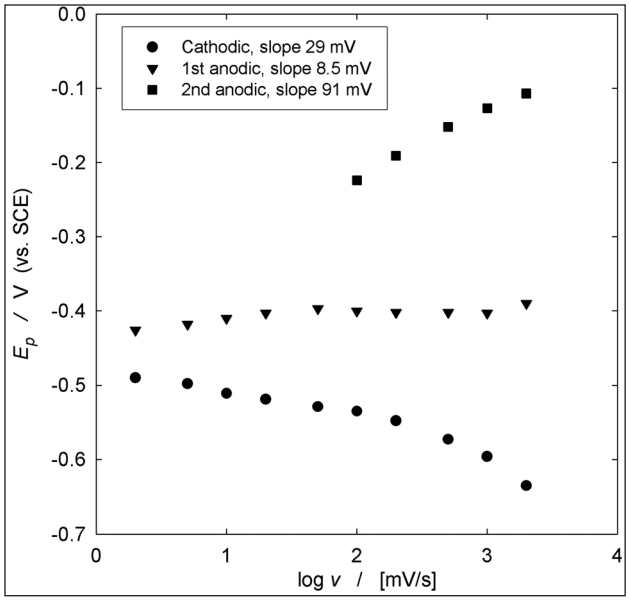

Fig. 3. Dependence of cathodic and anodic peak potentials on the logarithm of potential scan rate, as obtained from cyclic voltammograms

redox couple, some different values of E_m ranging from –0.186 to –0.210 V *vs.* RHE in pH-neutral solutions have been reported by different authors [2]. Thus, the value of E_m obtained in the present work for adsorbed riboflavin does not differ markedly from the data for dissolved riboflavin reported elsewhere.

At a higher potential scan rate exceeding 100 mV/s, the anodic peak splits into two separate peaks well defined at the highest scan rate applied up to 2000 mV/s (Fig. 1). For all scan rate values used, the sum of electric charge corresponding to both anodic peaks observed is nearly equal to the charge corresponding to the cathodic peak. This means that the splitting observed is rather of kinetic nature. Most probably, the two anodic peaks observed correspond to two consecutive one-electron oxidation steps of the adsorbed reduced form of riboflavin. If the charge transfer is fast relative to the potential scan (*i. e.* at a slow scan), the anodic oxidation of adsorbed reduced riboflavin appears as a one stage. In the opposite case, at a slow charge transfer relative to potential scan (*i. e.* at a fast scan), two separate oxidation processes are observed, corresponding to two one-electron transfer steps.

According to Laviron's model, charge transfer rate constants can be obtained for adsorbate from the difference of anodic and cathodic peak potentials [5]. An estimate for charge trasfer rate constant of 0.05 s^{-1} has been calculated from data obtained for riboflavin at a slow potential sweep. Earlier we have discussed the dependence of the charge transfer rate constant on the surface coverage, and concluded that the "true" values can be obtained only for a monolayer coverage [4]. For multilayer coverages, however, lower values are obtained because of a limited rate of charge propagation through a multilayer coverage. An estimate for charge transfer rate constant obtained here refers to a multilayer coverage of the electrode by adsorbed riboflavin, thus, following an analogy with a previous work [4], a "true" estimate should be up to one order of magnitude greater.

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ELEKTROCHEMINIS RIBOFLAVINO, ADSORBUOTO ANT GRAFITO ELEKTRODO, TYRIMAS

S a n t r a u k a

Riboflavino, adsorbuoto ant grafito elektrodo, elektrocheminiai redokso procesai tirti ciklinės voltamperometrijos metodu. Taikant didelį potencialo skleidimo greitį, nustatytos dvi atskiros redukuoto riboflavino anodinės oksidacijos stadijos. Nustatyta vidutinė redokso potencialo vertė (–0,460 ± 0,002 V pagal SKE pH 7,0 tirpale) ir krūvio pernašos greičio konstanta (0,05 s^{-1}).