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CATHODIC VOLTAMMETRY OF RIBOFLAVIN AT PLATINUM AND GOLD ROTATING DISC ELECTRODES

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ABSTRACT

The electro reduction of riboflavin (FMN) has been examined by cathodic rotating disc voltammetry in acidic and basic media at platinum and gold electrode. It has proved to be diversely reducible best at gold electrode in the alkaline medⁱa. Rapid voltammetric determination of the substance at gold electrode was effective and gave a mean standard deviation of 1%. Riboflavin at platinum electrode is cathodically active but the voltammogmms do not obey the Levich relationship and adsorption destroy the electrode activity. The technique can be conveniently applied to the rapid determination of riboflavin in pure solution and in formulated product.

Introduction

Riboflavin, vitamin 132, is an enzyme co-factor and a component of vitamin B-complex or multi-vitamin preparations. It plays an important role in biological oxidation-reduction processes and is found in plasma protein. Riboflavin is converted in the body to flavine mononucleotide (FMN) and then to flavine adenine dinucleotide (FAD). It is used in the treatment of ariboflavinosis (Reynold, 1982).

Considerable interest has been shown in the past to study the electrochemical reduction of riboflavin by polarography in aqueous and non-aqueous media. Its electrolytic reduction in aqueous media at dropping mercury electrode indicates two successive one electron steps to form the intermediate semiquinone radical. The completely reduced form with strong adsorption and reduction reaction occurs in approximately the same potential region where the molecule is strongly adsorbed (Kr, 1957). For a plot of the height of cathodic reduction wave against concentration the shape of the curve is similar to that of the Langmuir adsorption isotherm (Jambor, 1966). Polarography of riboflavin in dimethyl sulphoxide shows three reduction waves with the elimination of adsorption prewave which occurs in aqueous solution (Tatwawadi *of at*, 1968). The present investigation is designed to study the electrochemical reduction of riboflavin at the rotating disc electrodes.

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Materials and Methods

Riboflavin (FMN) was purchased from Sigma. The glassware, rotating electrode assembly, its operation and electronics, electrode activation procedure, reagents and preparation of solution have previously been described (Bishop and Hussein, 1984). The normal voltammetric scan rate was 5 mV s^{1} and the geometric area of the electrode was 0503 cm^2.

Results and Discussion

Voltammetry of riboflavin in acidic media

A $1x10^4$ mol 1^{-1} riboflavin in 0.1 mol 1^{-1} perchloric acid is reduced in a single cathodic wave at platinum disc electrode (Fig. la). The wave is shifted more cathodically and is merged with the solvent reduction wax as the electrode rotation speed is incased. At the heights rotation speed (50 Hz) the wave is diminished. This characteristic indicates that more adsorption occurs at higher rotation speeds poisoning the electrode surface with its reduction product. This is in agreement with the previous investigations conducted by polarography (Rader and Kaye, 1952 Kolthoff and litigant, 1952; Ke, 1957) and chronopotentiometry (Tatwawadi and Bard, 1964; Hartley and Chambers, 1964; Hartley and Wilson, 1964). Even in 10^{-5} mol 1^{-1} dilute solution the adsorption effect was considerable. Continuous sunning without pretreatment of the electrode resulted in disappearance of the reduction wave of riboflavin due to the presence of adsorbed special.

Typical curves of voltammogram for riboflavin at gold electrode are shown in Fig. lb. It depicts one reduction wave with a well defined limiting current. The wave shifted to more negative potential and merged with the back ground process with gradual increase of the rotation speed. A repeat scan without intervening activation of the electrode then shows total suppression of the reduction wave. Whereas with the 10^{-5} mol 1^{-1} solution the wave persists at an inactivated electrode but with a lower limiting current.

Voltammograms and frequency graphs are characteristic of adsorption, and disappearance of the reduction wax at an inactivated *electrode* identifies the adsorbate as the reduction product. At gold electrode Levich dependency (Bishop and Hussein, 1984) is displayed at low mass transport rate but is destroyed by product adsorption as the mass transfer rate increases on increasing the rotation speed.

Voltammetry of riboflavin to alkaline media

A $1x10^3$ mol 1^{-1} riboflavin at platinum electrode in 0.1 mol 1^{-1} sodium carbonate gives a single cathodic wave with well formed limiting current at lower rotation speeds (10-30 Hz) as shown in Fig. 1c. The wave progressively merges with the solvent wave as the rotation speed increases. Loss of wave height occurred on rescanning without intervening reactivation of the electrode.

The Levich plots of limiting current against square root of frequency are poor, suffering a change from straight line at lower rotation speeds (10-30 Hz) to a curvature at higher rotation speed (40-50 Hz), thus identifying the adsorption of reduction product. Whereas riboflavin at gold electrode shows one reduction wave with good limiting current. At higher rotation speed (40 and 50 Hz), two waves are evident from the Voltammograms (Fig. 1d). The appearance of two waves at higher frequency might indicate the formation of an intermediate product which is reduced at more negative potential. This is in agreement with the observations of Kemula *et al* (1958). A repeat scan at an inactivated electrode is almost unchanged so that in this medium at gold electrode deactivation of the electrode does not occur (i.e. no adsorption of reduced species). Limiting current bears a linear relationship to both square root of rotation speed and concentration with zero intercept. The half-wave potential either remains constant or increases marginally with increasing rotation speed and concentration.

The best performance for the reduction of riboflavin was achieved in 0.1 mol 1^{-1} sodium carbonate at gold electrode. Lingane and Davis (1944) have reported that a fairly good reduction wave of riboflavin is obtained in this medium.

Analytical Validity

A plot of limiting current versus concentration and rotation speed (Fig. 2) describes the calibration and Table 1 contains the regression analysis of calibration data at gold electrode. Assay of riboflavin was carried out using four solutions and the limiting current was measured at each of the five rotation speeds. The concentration was calculated from the slope and intercept of the calibration graph and the relative standard deviation of each set of five measurements is recorded in Table 1. The mean relative standard deviation is within 1% and less than 10 minutes are requ[i]red for each group of measurements. Thus the method is precise, rapid and convenient for the assay of riboflavin in pure solution and formulated products and is comparable to the pharmacopoeia) methods.

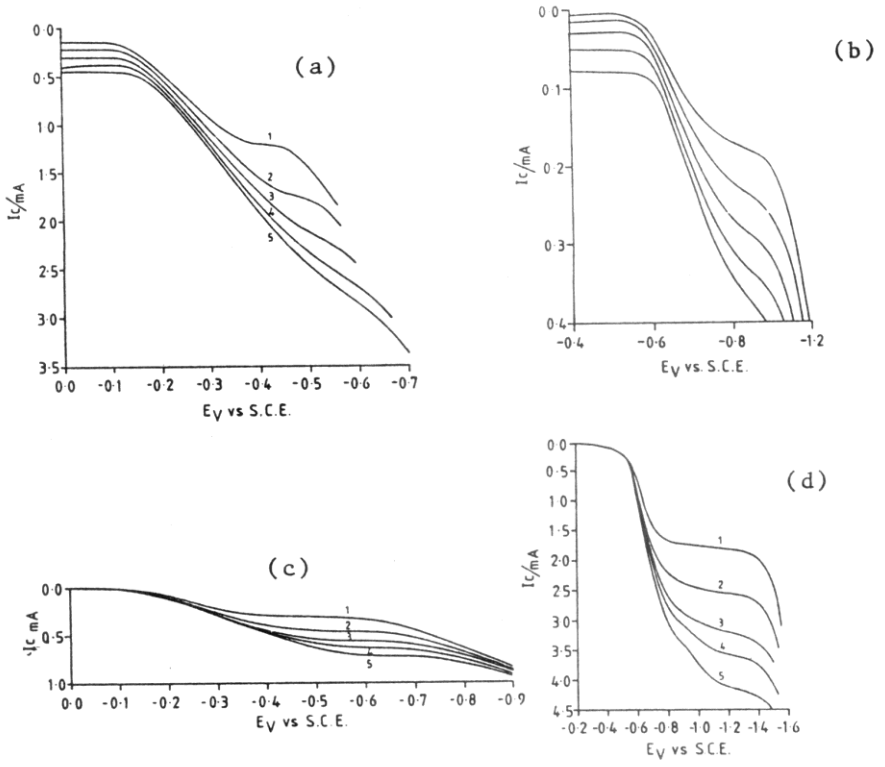

Fig. 1. Rotating disc electrode voltammograms of $5x10^{3}$ mol 1^{-1} riboflavin in 0.1 mol 1^{-1} perchloric add at (a) platinum and (b) gold electrode and in 0.1 mol 1^{-1} sodium carbonate at (c) platinum and (d) gold electrode. Electrode area 0503 cm^{-2}, scan speed 5 mVs^{-1}, temperature 25°C, nominal rotation speed for curves 1.5 are 10,20,30,40 and 50 Hz, respectively.

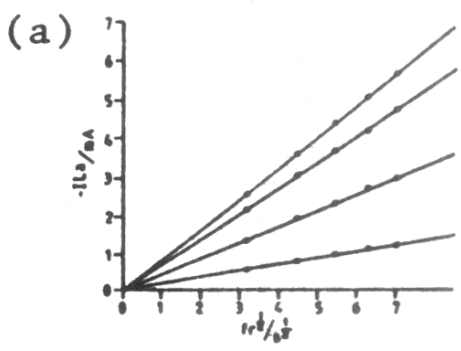

Fig-2(a) Levich plot of limiting current against square root of frequency of riboflavin in 0.1 mol 1^{-1} sodium carbonate at gold electrode.

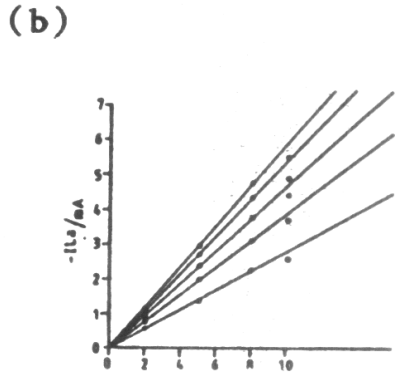

Fig. 2(b) Plot of limiting current against concentration of riboflavin in 0.1 mol 1^{-1} sodium carbonate at gold electrode, nominal concentration in order of increasing current i 0.2, 0.5, 0.8 and l x 10^4 mol 1^{-2}.

Table 1

Calibration results for riboflavin by RDE* at gold electorde. Electrode area 0.503 cm^2; medium 0.1 mol l^{-1} sodium carbonate; temperature 25°C.

Nominal frequency	Slope mA/ $mmol^{-1}$	Intercept mA	Correlation coefficient	S.D. of residuals mA	S.D. of slope/mA $l\ mmol^{-1}$	RSD of slope, %	Precision of determination conc., mmol l^{-1}	RSD %
5	0.40000	0.10000	1.00000	0.00000	0.00000	0.00	1.99984	0.96
10	0.52000	0.19000	1.00000	0.00001	0.00001	0.41	4.96251	0.41
20	0.65600	0.08601	0.99998	0.00004	0.00001	0.95	7.99942	0.96
25	0.75125	0.02412	1.00000	0.00015	0.00126	0.91	9.99649	0.98
30	0.80004	0.10000	1.00000	0.00000	0.00000	0.01		

*RDE = Rotating disc electrode

Influence of pH

The electroactive compound is examined in perchloric acid at pH 0 and 1 and in citrate-phosphate and sodium carbonate buffers at unit intervals from pH 2-7. The best reduction wave is obtained in 0.1 cool l'^1 sodium carbonate at both platinum and gold electrodes. The height of the wave decreases with an increase of pH and variation in the half-wave potential along with wave suppression at the highest rotation speed (50 Hz). No fresh wave appears and no improvement in wave height on increasing the pH is observed. In general the quality of the voltammogram deteriorates on increasing the pH upto 7. However, the disappearance of reduction wave at the highest rotation speed is due to enhanced mass transfer by adsorption. In all instances a second scan without intervening activation of the electrode showed complete disappearance of the wave in the pH range studied.

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