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Simultaneous Square-wave Voltammetric Determination of Riboflavin and Folic Acid in Pharmaceutical Preparations

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Abstract. The square-wave voltammetric (SWV) behaviour of riboflavin and folic acid was studied at a static mercury drop electrode by square wave voltammetry. In 0.05 *M* KCl (pH 5.89) a cathodic scan gave peaks at -0.56 and -0.87 V vs. Ag/AgCl for riboflavin and folic acid, respectively. The reduction peak currents are linearly dependent on the concentration of vitamins. Both vitamins can be simultaneously determined from the same voltammogram. The method proposed for the determination of riboflavin and folic acid in multivitamin tablets is very simple, rapid and does not involve time-consuming separation steps. The average contents of riboflavin and folic acid were found to be $14.8 \pm 1.26\%$ and $1.46 \pm 2.66\%$, for tablet A and $9.86 \pm 1.40\%$ and $1.47 \pm 2.0\%$ for tablet B, respectively.

Key words: square wave voltammetry, vitamins, riboflavin, folic acid.

Riboflavin [7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxypentyl) and folic acid {*N*-[4-(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl)amino)benzoyl]-L-glutamic acid} are parts of the vitamin B complex. Many methods for the determination of riboflavin and folic acid have been described in the literature: liquid chromatography (LC) with UV and fluorescence detection [1–3], spectrophotometry [4] and adsorptive stripping voltammetry [5–8]. These vitamins can be determined polarographically [9, 10]. Only a few attempts have been made towards the simultaneous determination of both vitamins [11, 12].

The polarographic behaviour of riboflavin has also been studied by Lindquist and Farroha [9] showing a $2e^-$, $2H^+$ reversible reduction process. Folic acid undergoes polarographic reduction showing three waves which have been studied previously [13, 15]. In acidic medium the first reduction wave corresponds to a $2e^-$, $2H^+$ reversible process of conversion of folic acid into 5,8-dihydrofolic acid and the second wave is due to a $2e^-$, $2H^+$ reductive cleavage of the latter dihydro derivative between the C-9 and N-10 positions to give 7,8-dihydro-2-amino-4-hydroxy-6-methylpteridine. Finally, the third wave is due to a $2e^-$, $2H^+$ reduction to the corresponding 5,6,7,8-tetrahydro derivative. Square-wave voltammetry allows good precision and selectivity for this type of determination. The theory shows that the voltammetric response can be affected by simultaneous reduction of other species. However, voltammetric reduction of other vitamins and constituents could not be very important and consequently the linear range should not be affected. In this study the simultaneous square-wave voltammetric behaviour of riboflavin and folic acid was studied and both vitamins were determined simultaneously in multivitamin preparations that contain other species at higher concentrations.

Experimental

Instrumentation

Square-wave voltammograms were recorded with a Princeton applied research (PAR) Model 384 B polarographic analyser equipped with a Houston Instrument DMP 40 (RE-0093) digital plotter. A PAR static mercury drop electrode (SMDE model 303) equipped with a saturated Ag/AgCl reference electrode and Pt-wire counter-electrode were used.

Experimental variables utilized as optimum values were as follows: initial potential -0.40 V; final potential -1.10 V, drop time 33.5 s, equilibration time 30 s, pulse height 0.04 V, scan rate 200 mV s^{-1}, scan increment 2 mV, frequency 100 Hz and medium drop size.

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All experiments were carried out at the ambient temperature (approximately 20 °C). Purified nitrogen gas was used to eliminate dissolved oxygen.

Reagents and Solutions

All reagents were of analytical-reagent grade. The vitamins (Sigma, pharmaceutical grade) were used as received without further purification. The supporting electrolyte was 0.05 *M* KCl solution (pH 5.89). Stock solutions of 2.66×10^{-4} *M* riboflavin and 3.4×10^{-4} *M* folic acid were prepared daily by dissolving the vitamins in triply-distilled water and protected from light and air. From these solutions, a series of solutions was prepared by serial dilution with deionized triply-distilled water. All the drug samples tested were fresh and purchased from a local pharmacy.

Procedure

A known volume of standard solution of vitamins was added to 10 ml of 0.05 *M* KCl solution (pH 5.89) in the polarographic cell, deaerated and the square-wave voltammogram recorded. A series of standard solutions at concentrations from 1×10^{-7}–1×10^{-5} *M* were used for riboflavin and folic acid. These two vitamins were also added to 10 ml of 0.05 *M* KCl (pH 5.89) in the polarographic cell at the same time and voltammograms were recorded simultaneously. The concentration dependencies of peak currents and detection limits were observed from these voltammograms.

Tablet Analysis

A multivitamin tablet was pulverized and a known weight (about 500 mg) was shaken with about 50 ml of distilled water and filtered through a Whatman No 41 filter-paper. The filtrate plus washing solution of the insoluble residue were diluted to 100 ml in a volumetric flask. A known volume of multivitamin tablet solution was taken to the polarographic cell for voltammetric analysis. The method of standard additions was used for quantitative determinations of the multivitamin tablets.

Results and Discussion

The most suitable supporting electrolyte for simultaneous SWV determination of some water-soluble vitamins (thiamin hydrochloride, riboflavin, calcium pantothenate, pyridoxine hydrochloride, folic acid, biotin, nicotinamide) was sought and various electrolytes (acetate, borate, potassium chloride, borate-potassium chloride, phosphate, potassium sulfate and bicarbonate-borate-phosphate buffer systems) were examined. For the simultaneous determination of these vitamins the peak potentials of vitamins must be separated from each other and current concentration relation must be linear. Potassium chloride supporting electrolyte was selected for the simultaneous SWV determination of the riboflavin and folic acid.

The effect of the concentration of this supporting electrolyte was also checked from 0.01 to 1.0 *M* KCl. 0.05 *M* KCl give the best response and therefore was chosen for all further measurements. Riboflavin showed a cathodic reduction peak at −0.56 V. Folic acid showed three cathodic peaks at pH 5.89 (0.05 *M* KCl) at −0.4, −0.87 and −1.2 V. One (−0.87 V) is well developed and provide the basis for quantitative determination of folic acid. The peak at −0.4 V shifted to more positive potentials as a result of the riboflavin present in the cell. Peak shapes and peak currents of two peaks at −1.2 V and at −0.4 V were not good enough for analytical purposes. The square-wave voltammograms of riboflavin and folic acid (peaks at −0.56 V and −0.87 V) are shown in Fig. 1. The effect of the pulse height on the peak current was tested. The peak height increased linearly for 0.02–0.15 V pulse heights, but the shape of the peak was not very good at 0.06–0.15 V pulse height. Therefore 0.04 V was chosen. Peak currents were little affected by scan rate, therefore a scan rate of 200 mV s^{-1}, with scan increment of 2 mV and a frequency of 100 Hz, was chosen for best sensitivity. Increasing the frequency of modulation (50–100 Hz) increased the peak currents, especially for riboflavin, (Fig. 2). Riboflavin and folic acid are known to adsorb on an SMDE. Therefore the equilibration time is critical for these two compounds. We observed that the peak currents for riboflavin and folic acid increased with increase of equilibration time. The peak height

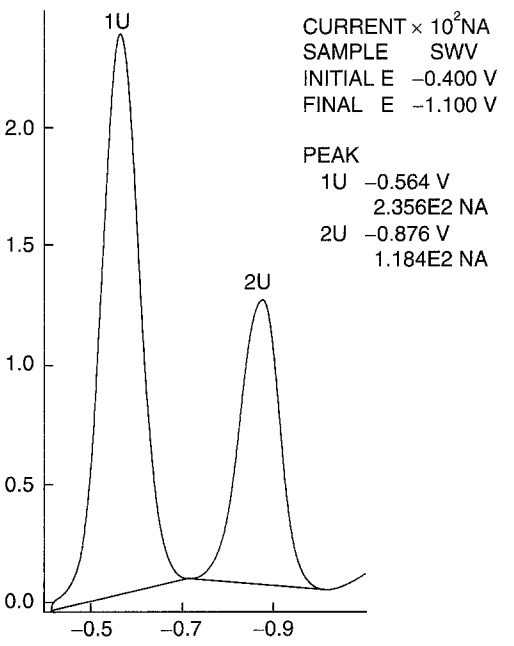

Fig. 1. Square-wave voltammograms of 2.6×10^{-7} *M* riboflavin (*1U*) and 3.4×10^{-7} *M* folic acid (*2U*) in 0.05 *M* KCl solution (pH 5.89)

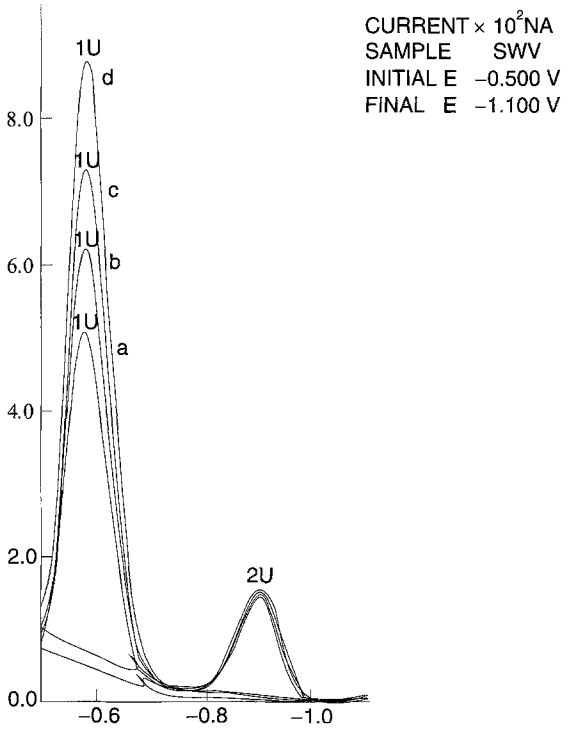

Fig. 2. Square-wave voltammograms of riboflavin (*1U*) and folic acid (*2U*) at different frequencies. *a* 50, *b* 80, *c* 90 and *d* 100 Hz

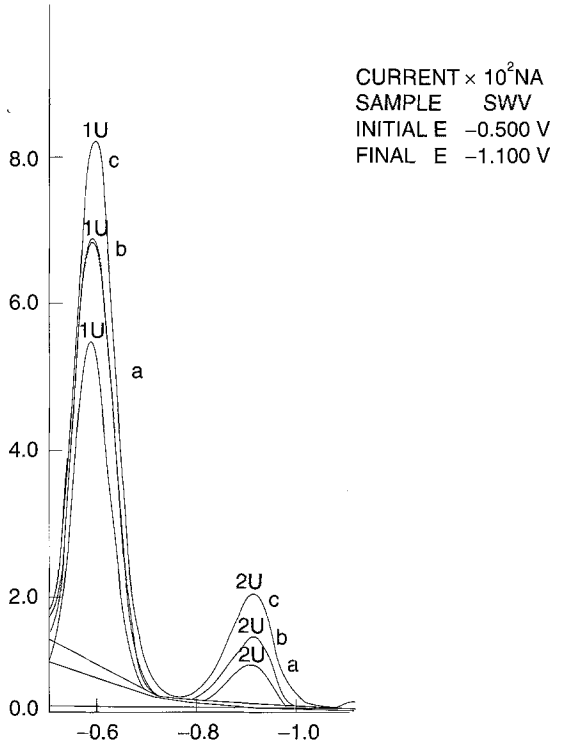

Fig. 3. Square-wave voltammograms of riboflavin (*1U*) and folic acid (*2U*) at different equilibration times: *a* 10, *b* 20 and *c* 30 s

increased linearly for equilibration times of 5–30 s, and was maximal at 30 s (Fig. 3). Therefore 30 s equilibration time was used for all measurements. Resolution of the peaks was good, as shown in Fig. 1. The cathodic reduction peaks of riboflavin and folic acid in 0.05 *M* KCl solution (pH 5.89) showed very good current/concentration linearity and good peak shapes. The current/concentration dependencies were linear in the 1×10^{-7}–1×10^{-5} *M* concentration range for both riboflavin and folic acid. The calibration graphs employed had the following equations: $i_p = 3.74 \times 10^8 C + 124$ (for riboflavin) and $i_p = 1.06 \times 10^8 C + 79$ (for folic acid). Both of the graphs had high correlation coefficients ($r = 0.998$).

Cyclic voltammetry was used to test the reversibility of the reductions of these two vitamins and both gave reversible reduction behaviour.

Analytical Application in Tablets

Simultaneous determination of riboflavin and folic acid was investigated in two types of multivitamin tablet chosen from the pharmacy markets. The SWV voltammogram of the solutions of these tablets (0.05 *M* KCl, pH 5.89) are shown in Fig. 4. Separated and

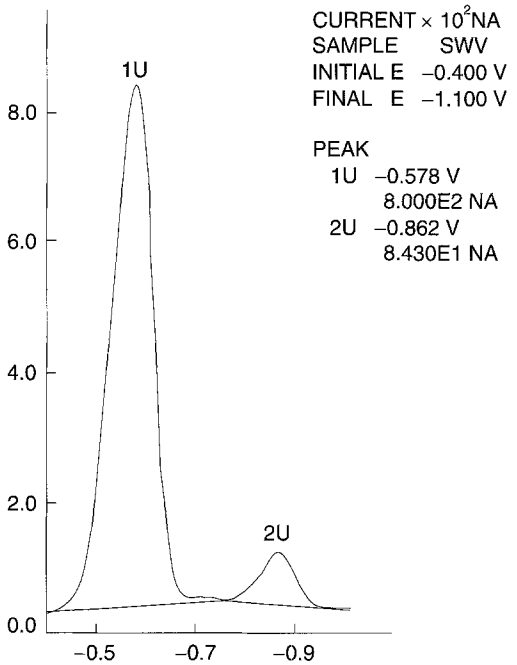

Fig. 4. Square-wave voltammogram of tablet solution in 0.05 *M* KCl solution (pH 5.89) riboflavin (*1U*) and folic acid (*2U*)

well-defined peaks were observed for two vitamins in the −0.4 to −1.1 V potential range. Voltammetric reduction of other vitamins and constituents were not

Table 1. Results for SWV determination of the vitamins in two tablets

Multivitamin tablets	Vitamins	Specified (mg)	Found[a] (mg)	S.D. (mg)	R.S.D (%)
A	Riboflavin	15.0	14.8	0.19	1.26
	Folic acid	1.5	1.46	0.04	2.66
B	Riboflavin	10.0	9.86	0.14	1.40
	Folic acid	1.5	1.47	0.03	2.0

[a] Mean of five determinations.

Table 2. Recovery of riboflavin and folic acid in mixture of vitamins solution

Vitamin	Added (mg)	Found[a] (mg)	RSD (%)
Riboflavin	10.0	9.8	2.0
Folic acid	1.5	1.48	2.0

[a] Mean of five determinations.

found in this range, but the linearity range and peak potentials were affected somehow by the other ingredients present in multivitamin tablets. Tablet A containing 15 mg of riboflavin and 1.5 mg of folic acid and tablet B containing 10 mg of riboflavin and 1.5 mg of folic acid were analysed (five samples of each). The standard addition method was used to determine the contents of both vitamins. The results obtained are given in Table 1. To investigate any interaction between the analyte and matrix, recovery tests were performed on a synthetic sample prepared in the laboratory to match the tablets in vitamins and excipients. This mixture was analysed by using the standard addition method and the results are given in Table 2. The mean recovery was 98% and the relative standard deviation (RSD) was in the range of 2.0%.

Conclusions

The proposed method has many advantages. SWV is valuable technique for simultaneous determination of folic acid and riboflavin in the presence of other constituents with good accuracy and precision. This technique could be satisfactorily applied for determination of these vitamins in multivitamin preparations. The method is much faster and cheaper than the other methods.

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