Simultaneous Square-Wave Voltammetric Determination of Thiamine Hydrochloride, Riboflavin, Folic Acid and Nicotinamide in Multivitamin Preparations

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Summary-Using square- wave voltammetry at a static mercury drop electrode, a fast and simple method has been developed for the assay of the water-soluble vitamins thiamine hydrochloride, riboflavin, folic acid and nicotinamide. In Britton Robinson buffer (pH 9.5) by cathodic scan thiamine hydrochloride give a peak at -1.33 V, riboflavin give two peaks at -0.61 V and -0.73 V, folic acid give a peak at -0.91V and nicotinamide give a peak at -1.74 V vs. Ag/AgCl reference electrode at the same buffer solution. These four vitamins can be determined by the same voltammogram. The reduction peak currents are linearly dependent on the concentration of the vitamins. The method is successfully applied to determine these four vitamins contents of multivitamin preparations simultaneously. The values found agreed well with those determined by other methods.

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INTRODUCTION

Thiamine hydrochloride [3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride] riboflavin [7,8- dimethyl-10-(D-ribo 2,3,4,5-tetrahydroxypentyl) isoalloxazine] and folic acid [N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl)-L-glutamic acid] are part of the vitamin B complex (vitamin B_1, vitamin B_2 and vitamin B_c, recpectively) and nicotinamide[3-pyridine-carboxylic acid amide] is known as niacinamide. Many methods for the determination of these water soluble vitamins using different physical, chemical and biological methods have been published,[1-3] but the determination of vitamins in pharmaceutical preparations is often complicated because of the large excess of other ingredients. Some of these methods depend on the use of titrimetry,[4] complexometry,[5] spectrophotometry,[6-8] HPLC[9-11] and adsorptive stripping voltammetry,[12,13] but these techniques have never been used for the simultaneous direct determinations of these four vitamins in pharmaceutical preparations. Vitamins can be determined by polarographic methods, especially vitamin C[14,15] and vitamin E[16] were studied extensively. Folic acid, riboflavin and ascorbic acid have been determined simultaneously using anodic polarography by Kruze[17] in the borate-phosphate buffer solution. Simultaneous adsorptive stripping voltammetric determination of folic acid and riboflavin in multivitamin preparations was studied in 0.1 M sodium acetate buffer.[1] Folic acid was determined in pharmaceutical preparations by adsorptive stripping voltammetric[18] method. The polarographic reduction behaviour of riboflavin has been studied by d. c. Polarography showing a two-electron reversible reduction process.[14] Thiamine, riboflavin, nicotinamide and ascorbic acid have been determined using differential pulse polarography and linear-sweep voltammetry at a carbon-paste electrode.[19]

Square-wave voltammetry (SWV) allows good precision and selectivity. The theory shows that the voltammetric response can be affected by simultaneous reduction of other species. However, if the current response from the reduction of other vitamins and constituents is negligible, the linear range should not be affected.[1]

Using square wave voltammetry at a static mercury drop electrode, a fast and simple procedure is proposed in this paper. Thiamine hydochloride, riboflavin, folic acid and nicotinamide were successfully determined in multivitamin preparations containing other species at higher concentrations.

EXPERIMENTAL

Apparatus

A Princeton Applied Research (PAR) Model 384 B polarographic analyser equipped with a Houston Instrument DMP 40 (RE-0093) x-y plotter was used to measure and record the square-wave voltammograms. A PAR static mercury drop electrode system (SMDE model 303) equipped with a saturated Ag/AgCl reference electrode and Pt-wire counter electrode were used. .

Reagents and Solutions

All reagents used were analytical-reagent grade. The vitamins (pharmaceutical grade) were used as received without any further purification. The supporting electrolyte was Britton-Robinson(BR) buffer solution (pH 9.5). Stock solutions of $8x10^{-5}$ M vitamin B_1, vitamin B_2, vitamin B_c and $8x10^{-3}$ M nicotinamide were prepared daily by dissolving the vitamins in triple-distilled water and protected from the light and the air oxygen. From this solutions a series of solutions was prepared by serial dilution with deionised triple-distilled water. All drug samples tested were purchased from the local pharmacy.

Procedure

A known volume of the standard solution of vitamin was added to 10 ml of BR buffer solution (pH 9.5) in the polarographic cell, deaerated and square-wave voltammograms were recorded for each sample. Working parameters were selected as static mercury electrode drop time of 12.2 s equilibrate time 5 s, pulse height 0.030 V, drop size medium, scan rate 200 mVs^{-1} scan increment 2 mV and frequency 100 Hz. All measurements were carried out at the ambient temperature (approximately 20 °C). The initial potential of scanning was -0.500 V and final potential was -1.850 V vs. Ag/AgCl reference electrode. Dearation of solutions in the cell was made by purging purified nitrogen through the solution and nitrogen was also used to blanket the surface of the solution.

Calibration graphs were made by the addition of known volume of the stock solution of $8x10^{-5}$ M the vitamin B_1, B_2, B_c and $8x10^{-3}$ M of the nicotinamide to the buffer solution in the cell. All of these four vitamins were also added to 10 ml BR of buffer solution (pH 9.5) in polarographic cell at the same time and voltammograms of vitamin mixtures were recorded simultaneously. The concentration dependence of peak currents and detection limits were analysed from these voltammograms.

Multivitamin Tablets Analysis

A multivitamin tablet was pulverized and a known weight was shaken with about 50 ml of water for 5 min. and filtered through a Whatman No 41 filter-paper. The filtrate and washing solutions were diluted to 100 ml in a calibrated volumetric flask. A known volume of this multivitamin tablet solution was added to 10 ml of BR buffer solution (pH 9.5) in the polarographic cell for voltammetric analysis. The voltammograms were recorded at the beginning and after adding appropriate amounts (50-200 µl) of stock standard solutions of vitamins $4x10^{-4}$ M

of vitamin B_1, 3.4×10^{-4} M of vitamin B_2, 5×10^{-4} M of vitamin B_c and 8×10^{-2} M of nicotinamide The standard addition method was used for quantitative determinations of these vitamins in multivitamin tablets.

The comparison values were obtained by the following methods: Adsorptive Stripping Voltammetry[1] for riboflavin and folic acid, thiochrome method[20] for thiamine and Differential Pulse Polarography[14] for nicotinamide.

For complete simulation of the vitamin tablets, synthetic samples containing the mixture of vitamins and all the other components were prepared and these synthetic samples were used for adequate recovery study.

RESULTS AND DISCUSSION

The most suitable buffer solution for the simultaneous SWV determination of the water soluble vitamins (thiamine hydrochloride, riboflavin, calcium pantothenate, pyridoxine hydrochloride, folic acid, biotin, nicotinamide) was investigated. Britton- Robinson (BR) buffer solution was selected and SWV voltammograms of all these vitamins were taken at different pH values between 6 and 11. Only vitamin B_1, vitamin B_2, vitamin B_c and nicotinamide gave simultaneous cathodic reduction peaks in BR buffer at pH 6.5-9.5. The effect of pH on the polarographic waves was investigated. The reduction of vitamin B_c[21], nicotinamide[14] and vitamin B_1 are well defined at pH 8 and above. Vitamin B_2 sensitive to alkaline media, but polarographic determination of vitamin B_2 has been worked in 0.001 M NaOH[23] and in BR (pH 11.98) buffer solution[24]. The peak potentials of these four vitamins must be sufficiently separated from each other for the simultaneous determination. It is found that pH 9.5 (Britton-Robinson buffer) was the most appropriate medium to separate these peaks from each other. Vitamin B_1 shows a cathodic reduction peak at -1.33 V (v.s. Ag/AgCl). This peak shows very good current-concentration linearity and

good peak shape. Vitamin B_2 shows two cathodic peaks at -0.61 V and -0.73 V. Both of the peaks show good current-concentration linearity and good peak shape. The first peak at -0.61 V was chosen for the current-concentration relation. The SWV voltammograms show a cathodic peak at -0.91 V for folic acid and at-1.74 V for nicotinamide. The influence of frequency and equilibration time on the peak heights were investigated. Comparative measurements of these four vitamins showed that increasing frequency results with higher currents for the reduction of vitamin B_2 and B_c until 100 hz frequency increasing the frequency from 100 to 150 Hz caused only little change in the peak current. Frequency of 100 Hz and scan rate of 200 mVs^{-1} were selected for all measurements.

Vitamin B_2 and B_c are known to adsorb on the static mercury drop electrodes[25] the equilibration time is critical for these two compounds. Therefore the accumulation curves of vitamin B_2 and B_c ($1x10^{-6}$ - $2x10^{-5}$ M), in the presence of the rest of compounds, were investigated to obtain an adequate evaluation of the equilibration time (5 - 30 s). It is noticed that peak currents are increased for these two vitamins with increase of equilibration time but peak currents of vitamin B_1 and nicotinamide are decreased with increase of equilibration time. Therefore equilibration time of 5 s was selected for all measurements. Resolution of peaks was good as shown in Fig. 1. The current concentration dependencies were linear in $4x10^{-7}$ - $2x10^{-5}$ M concentration range for vitamin B_1, B_2 and B_c. Nicotinamide currents were linear and correlation coefficient values were good in $4x10^{-5}$ - $1x10^{-3}$ M concentration range (Table 1).

The cyclic Voltammetry (CV) technique was showed that the vitamin B_2 has reversible reduction behaviour.

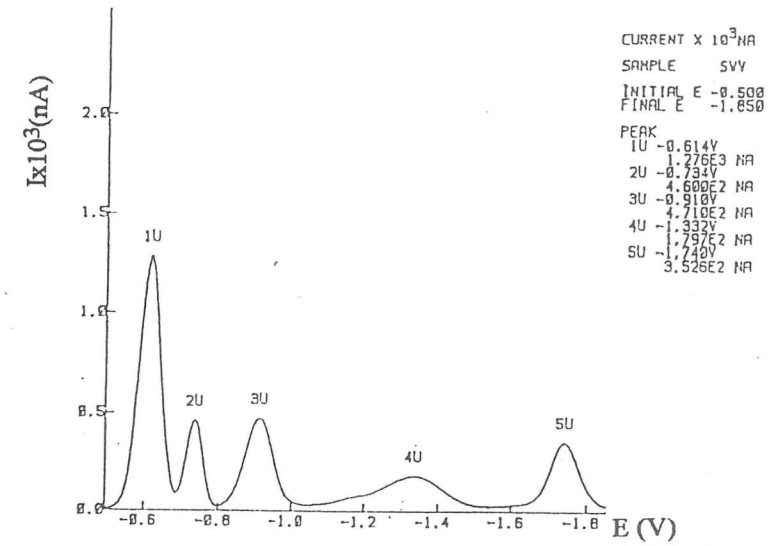

Fig. 1. Square-wave voltammogram of vitamin B_2 (*1U, 2U;* $1.7x10^{-6}$ M) vitamin B_c (*3U;* $2.5x10^{-6}$ M). Vitamin B_1 (*4U;* $1.9x10^{-6}$ M) and nicotinamide (*5U;* $4x10^{-4}$ M) in BR buffer solution (pH 9.5).

TABLE 1

Characteristic features of calibration plots for some vitamins

Vitamins	Equation	Correlation coefficient	Limit of linearity/M
Vitamin B_1	$y = 91.17 + 2.62.10^7x$	0.997	4.10^{-7} - 3.10^{-5}
Vitamin B_2	$y = -220.78 + 8.75.10^8x$	0.998	4.10^{-7} - 2.10^{-5}
Vitamin B_c	$y = 43.09 + 1.52.10^8x$	0.997	4.10^{-7} - 2.10^{-5}
Nicotinamide	$y = 41.93 + 7.03.10^5x$	0.998	4.10^{-5} - 1.10^{-3}

Applications of the Method to Drug Formulations

Simultaneous determination of the vitamin B_1, vitamin B_2, vitamin B_c and nicotinamide was investigated in three types multivitamin tablet chosen from the pharmacy markets. The SWV voltammogram of the multivitamin tablet solution in BR, buffer (pH 9.5) is shown in Fig. 2. Separated and well defined peaks were observed for these four vitamins in the -0.500 to -1.850 V potential range. Voltammetric reduction of other vitamins and constituents were not found in this range in this buffer solution but the peak potentials were affected somewhat by the other ingredients present in multivitamins tablets. The standard addition method is used to determine the amount of these four vitamins content of the tablets. The results of analysis are given in Table 2.

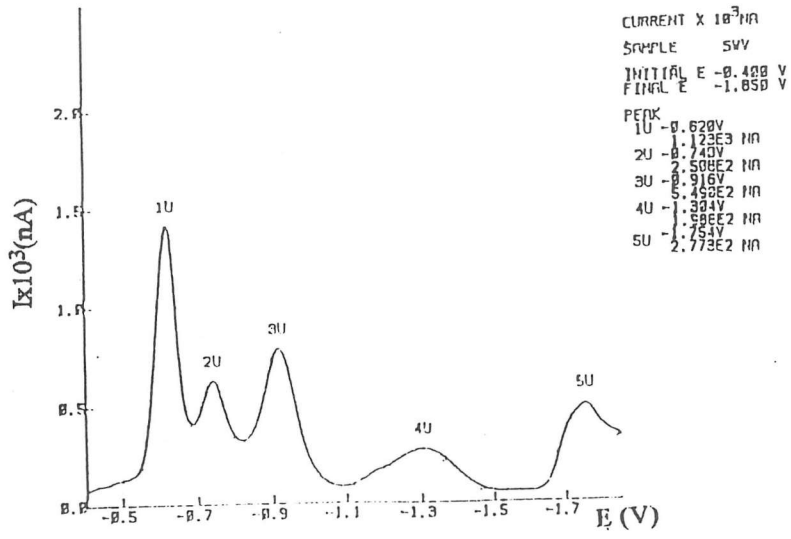

Fig. 2. Square-wave voltammogram of multivitamin tablet solution in BR buffer (pH 9.5). Vitamin B_2 (*1U, 2U*) vitamin B_c (*3U*). Vitamin B_1 (*4U*) and nicotinamide (*5U*).

TABLE 2

Vitamin contents of some preparations (all values in mg/tablet)

Multivitamin	Vitamin B_1			Vitamin B_2			Vitamin B_c			Nicotinamide		
tablets	dec.	f. v.	f. r. m.	dec.	f. v.	f. r. m.	dec.	f. v.	f. r. m.	dec.	f. v.	f. r. m.
Tablet A	250.0	225 ± 15	228	-	-	-	-	-	-	-	-	-
Tablet B	25.0	24±2	20.	10	9.8±0.1	9.3	1.5	1.6 ± 0.1	1.6	100	98±2	99
Tablet C	15.0	14.7±0.2	15	15	14.8±0.4	14.7	1.5	1.3 ± 0.1	1.7	50	47±4	48.

The results are the average of five determinations.

dec: Declared value.

f. v.: Found voltammetically.

f. r.m.: Found reference method.

The values are slightly lower than the specified values. The reason may be the decomposition or degradation of some of the vitamin contents after the production date. A comparison between the SWV and reference methods (Adsorptive Stripping Voltammetric[1], Differential Pulse Polarography[14], thiochrome method[20]) is given in Table 2. These methods gave approximately similar results. To investigate any interaction between the vitamins and other components, recovery tests were performed on the synthetic samples prepared in the laboratory composed of the content of these multivitamin tablets. This mixture was analysed by using standard addition method and the results are given in Table 3. The mean recovery observed was 98% and the relative standard deviation (RSD) was about of 2%.

TABLE 3

Recovery of Vitamin B_1, B_2, B_c and Nicotinamide in Mixture of Vitamins Solution

Vitamins	Vitamin contents of solution (mg)	Vitamin found* (mg)	RSD (%)
Vitamin B_1	25.0	24.6	1.6
Vitamin B_2	10.0	9.8	2.0
Vitamin B_c	1.5	1.5	0.0
Nicotinamide	100	97.7	2.3

* The results are the average of five determinations

CONCLUSIONS

The SWV method developed for the simultaneous determination of vitamin B_1, vitamin B_2, vitamin B_c and nicotinamide are rapid, sensitive and accurate method for the separate and simultaneous determination of these vitamins in the solution or in drug formulations

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