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Short communication

Voltammetric determination of vitamins in a pharmaceutical formulation

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Abstract

Direct current polarography and differential pulse polarographic methods have been developed for the qualitative as well as quantitative analysis of vitamin B_1, B_2 and B_6. Thiamin (Vitamin B_1) produced a well-defined wave in 0.1 M KCl at pH 5.2 with $E_{1/2} = -1.2$ V and $E_p = -1.22$ V versus saturated calomal electrode (SCE). Riboflavin (Vitamin B_2) gave two distinct waves in Britton Robinson buffer at pH 1.8 with $E_{1/2}$ values $= -0.13$ and -0.34 V versus SCE and at pH 6.5 with $E_{1/2} = -1.10$ V and $E_p = -1.2$ V versus SCE. Pyridoxin (Vitamin B_6) produced a well-defined wave in Britton Robinson buffer at pH 6.5 with $E_{1/2} = -1.7$ V and $E_p = -1.68$ V versus SCE. All the three Vitamins under study are reversibly reduced at the electrode surface. The number of electrons involved in the electrode process for vitamin B_1 and B_6 is one in each case where as for the two waves of B_2 it is one and two, respectively. This has been confirmed by the measurement of $E_{3/4}-E_{1/4}$ values and also from the log plot slopes for the reduction waves. The wave height of polarogram was found to be proportional to the vitamin concentration. The developed methods have been standardised for the determination of these compounds in pharmaceutical formulation. The concentration of Vitamin B_1, B_2 and B_6 are found to be 9.96, 9.92 and 3.01 mg, respectively in 240 mg of capsule powder of a standard company (name has not been disclosed due to secrecy purpose). The results have been found to be in excellent agreement to that claimed by the manufacturer. The observed data has been subjected to statistical analysis, which revealed high reliability and precision.

Keywords: Vitamins; Thiamin; Riboflavin; Pyridoxin; DCP; DPP; Pharmaceutical formulation

1. Introduction

Vitamins are organic compounds which are required for the normal growth and maintenance of life of animals, including man, who, as a rule are unable to synthesise these compounds by anabolic processes. These compounds are essential for the transformation of energy and for the regulation of metabolism of structural units [1].

Although an up-to-date survey of literature reveals that the determination of vitamins has been

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carried out using various methods like chromatography, coulometry, spectrophotomery, etc. [2–8], but looking at the extra ordinary detection sensitivity, oligo determination capability, non-destructive nature, simplicity, and accuracy of polarographic method of analysis, this method is widely being used by analysts for the analysis of organic compounds in samples of different origin. Most of the vitamins produce reasonable polarographic/voltammetric responses and can be qualitatively as well as quantitatively determined voltammetrically [9–12], without interference of the presence of each other and of other species under optimum experimental conditions.

The authors have therefore standardized DCP and DPP methods for the analysis of vitamin B_1, B_2 and B_6 and used the developed procedure for the analysis of these vitamins in a pharmaceutical-formulation, i.e. a multivitamin capsule, the results of which have been reported in the paper. The composition of the capsule is as follows:

Thiamine Mononitrate I.P.	10 mg
Riboflavin I.P.	10 mg
Pyridoxin Hydrochloride I.P.	3 mg
Vitamin B_{12} I.P. (as stablets 1:100)	5 mcg
Niacinamide I.P.	50 mg
Calcium Pantothanate I.P.	12.5mg
Folic acid I.P.	1 mg
Ascorbic acid I.P.	150 mg
(per 240 mg of capsule powder)	

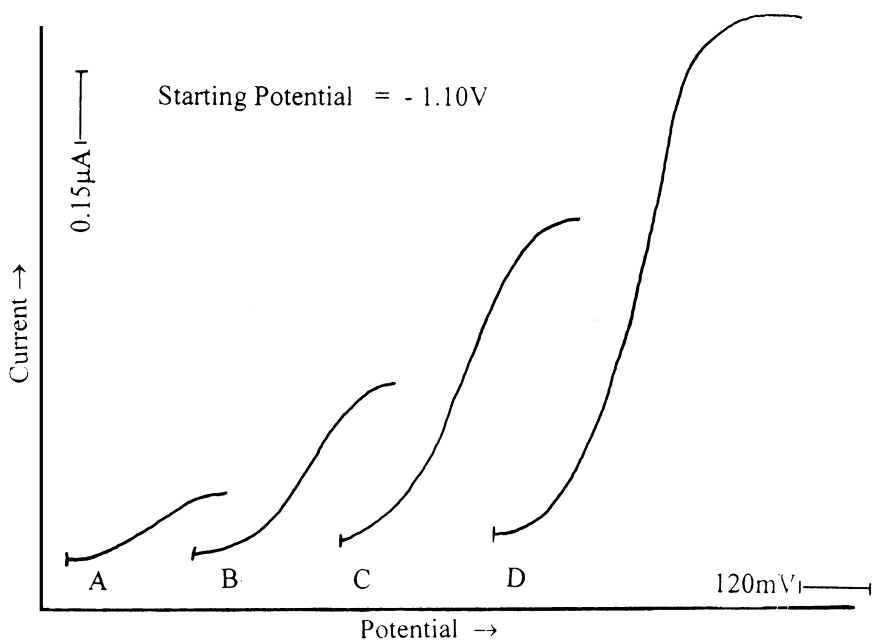

Fig. 1. Effect of concentration on DC polarograms of Vitamin B_1 in 0.1 M KCl at pH 5.2. (A) 3.3 mg, (B) 6.6 mg, (C) 9.9 mg, (D) 13.2 mg (per 50 ml of analyte).

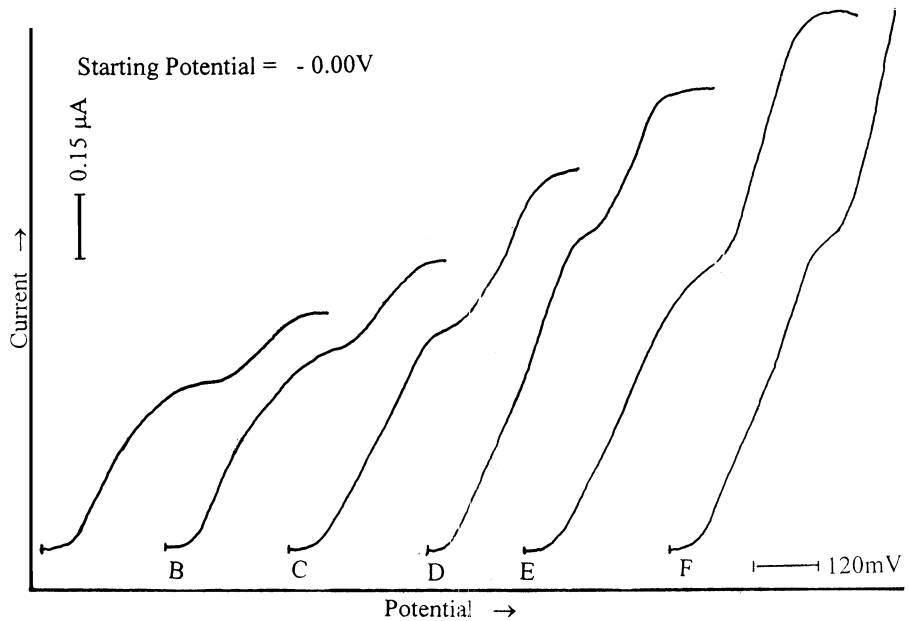

Fig. 2. Effect of concentration on DC polarograms of Vitamin B_2 in BR buffer at pH 1.8. (A) 4.5 mg, (B) 9.0 mg, (C) 13.5 mg, (D) 15.0 mg, (E) 27.0 mg, (F) 31.5 mg (per 50 ml of analyte).

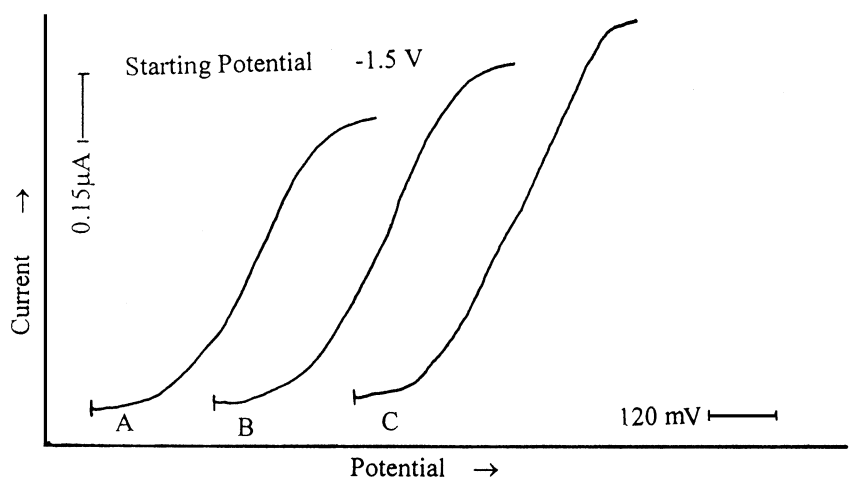

Fig. 3. Effect of concentration on DC polarograms of Vitamin B_6 in BR buffer at pH 6.5. (A) 1.6 mg, (B) 3.2 mg, (C) 4.8 mg (per 50 ml of analyte).

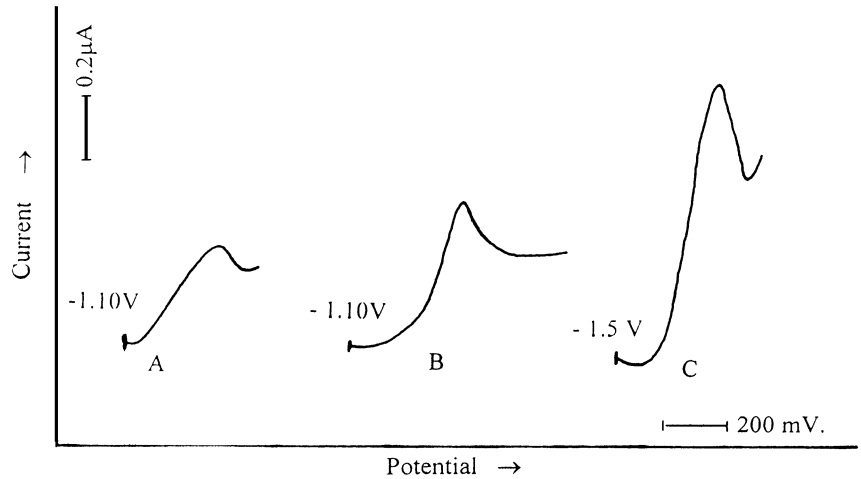

Fig. 4. Differential pulse polarograms of (A) Vitamin B_1 (13.2 mg) in 0.1 KCl at pH 5.2. (B) Vitamin B_2 (7.2 mg) in BR buffer at pH 6.5. (C) Vitamin B_6 (12.6 mg) in BR buffer at pH 6.5 (per 100 ml of analyte).

Table 1
Results[a] on multivitamin capsule for its vitamin B_1, B_2 and B_6 content (mg/240 mg of capsule powder)

Vitamin	Parameter amount	DCP		DPP	
		Added	Found	Added	Found
		–	9.96	–	10.60
		9.90	19.84	9.90	20.30
B_1	%R	99.8%	99.0%		
	RMD	0.001	0.006		
	S.D.	0.2	0.1		
	CV	2.00	0.94		
	Amount	–	9.92	–	10.80
		10.8	20.70	10.80	21.40
	%R	99.8%	99.0%		
B_2	RMD	0.001	0.005		
	S.D.	0.094	0.1		
	CV	0.94	0.92		
	Amount	–	3.01	–	3.15
		4.8	7.80	4.8	7.92
	%R	99.9%	99.6%		
B_6	RMD	0.002	0.02		
	S.D.	0.1	0.1		
	CV	3.32	3.17		

[a] Average of four determinations.
% R = recovery; RMD = relative mean deviation; S.D. = standard deviation; %CV = coefficient of variance.

Table 2
Final analysis results on multivitamin capsule for its vitamin B_1, B_2 and B_6 content (mg/240 mg of capsule powder)

Compound	Reported by the manufacturer	Found	
		DCP	DPP
Vitamin B_1	10.0	9.96	10.60
Vitamin B_2	10.0	9.92	10.80
Vitamin B_6	3.0	3.01	3.15

2. Experimental

2.1. Apparatus

The DCP and DPP studies were carried out on an Elico (India) DC polarograph, model CL-357 and Elico pulse polarograph, model CL-90, respectively. Systronic digital pH meter-335 was used for pH measurements. Polarographic cell consisted of a three electrodes assembly with a calomal electrode (reference electrode) and platinum electrode (auxiliary electrode). The working electrode for DCP and DPP was dropping mercury electrode. The capillary characteristics of the DME had a $m^{2/3}\,t^{1/6}$ value of 2.5 $mg^{2/3}\,s^{-1/2}$ at 60 cm effective height of mercury column.

2.2. Solutions

The chemicals used were of Anala R/BDH grade. Doubly distilled water was used to prepare the solutions. A total of 0.01 M solutions of vitamin B_1 (Thiamin) (LOBA), vitamin B_6 (Pyridoxin) (ROCHE) and 1 M potassium chloride KCl were prepared by dissolving a requisite quantity of the compound/salt in doubly distilled water. A total of 0.01 M vitamin B_2 (Riboflavin) (LOBA) solution was prepared by dissolving the requisite amount in 5 ml of 0.1 N NaOH. Then the solution was made up to 100 ml with distilled water. Britton Robinson (BR) Buffer was prepared by mixing 0.04 M phosphoric acid, 0.04 M Acetic acid and 0.04 M boric acid. The pH adjustments were made as described [14]. The test solutions were deaerated by bubbling purified Hydrogen gas for 10 min before recording the polarogram/voltammogram.

2.3. Determination of Thiamin

Different concentrations of thiamin were taken in the polarographic cell containing 5 ml of 1 M KCl. The volume was made to 50 ml with distilled water. The pH of the analyte was adjusted to 5.2 using HCl/NaOH solution. The polarogram was recorded as described earlier.

For the determination of thiamin in the pharmaceutical formulation, 40 mg of the capsule powder was dissolved in 10 ml of distilled water. The solution was then transferred to polarographic cell containing 5 ml of 1 M KCl and the volume was made up to 50 ml. The pH of the test solution was adjusted to 5.2 and the polarogram/voltammogram was recorded as earlier.

2.4. Determination of Riboflavin

Different concentrations of Riboflavin were taken in the polarographic cell containing 5 ml of BR buffer at pH 1.8. The volume was made to 50 ml with distilled water and the polarogram/voltammogram was recorded as earlier.

The wave thus produced by Riboflavin is however not useful for the determination in pharmaceutical formulation because at this pH the wave of Riboflavin is suppressed due to the presence of other compounds in the sample. Hence, the determination in the formulation was carried out in BR buffer at pH 6.5. The wave produced at this pH at $E_{1/2}/E_p = -1.10$ V/ -1.2 V versus saturated calomal electrode (SCE) is though a distorted one, yet its height is proportional to the concentration of compound in the solution.

For the determination of Riboflavin in pharmaceutical formulation, capsule powder was dis-

solved in 5 ml of 0.1 N NaOH and the solution was diluted to 10 ml with distilled water. The solution was then transferred to the polarographic cell containing 5 ml BR buffer. The volume was made up to 50 ml and the pH was adjusted to 6.5. Polarogram/voltammogram was recorded as earlier.

2.5. Determination of Pyridoxin

Different concentrations of vitamin B_6 were taken in the polarographic cell containing 5 ml of BR buffer at pH 6.5. The volume was made to 50 ml with distilled water. Polarogram/voltammogram was recorded as described earlier.

For the determination of pyridoxin in pharmaceutical formulation, 40 mg of capsule powder was dissolved in 10 ml of distilled water. The insoluble matter was filtered off. Solution was then transferred to the polarographic cell containing 5 ml of BR buffer and the volume was made up to 50 ml. The pH was adjusted to 6.5. Polarogram/voltammogram was recorded as earlier.

3. Results and discussion

Under the above mentioned experimental conditions, vitamin Bs are easily reducible at DME surface. Vitamin B_1 gives a well-defined wave with $E_{1/2} = -1.2$ V[13] (Fig. 1) and $E_p = -1.22$ V versus SCE (Fig. 4). The electrode process was found to be reversible. The number of electrons involved in the electrode process was found to be one, as calculated by measuring $E_{3/4} - E_{1/4}$ values. This has been confirmed by the log plot slopes too.

Vitamin B_2 produced two distinct waves with $E_{1/2}$ values $= -0.13$ and -0.34 V versus SCE [14]. Both the waves were found to be reversible reduction waves and the number of electrons being one and two respectively (Fig. 2). Vitamin B_6 produced a well-defined reversible wave with $E_{1/2}$ value $= -1.7$ V (Fig. 3) and $E_p = -1.68$ V (Fig. 4) versus SCE. The number of electrons involved in the electrode process was found to be one.

The standardized methods were found to be very accurate for the determination of these compounds in pharmaceutical formulations. Quantitative analysis of the sample has been done by wave height and the statistical analysis of the results by external spiking method. The percentage recovery in all the cases was found to be more than 99.7% (Table 1). Replicate analysis of the analyte was done to calculate statistical data, i.e. Standard deviation and coefficient of variance (Table 1), which never exceeded 0.2 and 3.3, respectively. Thus confirming the reliability of the determination and also the stability of the analyte in solution and during the actual analysis.

The final analysis results (Table 2) have been compared with those claimed by the manufacturer, on the basis of absorption analysis of the formulation [15,16] and were found to be in good agreement. The results show that the DCP method is more accurate for the determination of vitamins in the sample because due to the higher minimum detection limits of DPP, other compounds present in the sample also produce DPP signals close to the signal of the vitamins. As such, under the given conditions the DPP signal due to vitamin is distorted, which makes an accurate determination of the vitamin difficult using DPP mode. Looking at the extra ordinary detection sensitivity, oligo determination capability, non-destructive nature, simplicity, accuracy and rapidity of the above discussed method, this method may be recommended in lieu of the prevalent methods like chromatography, spectrophotometry, etc. The DC polarographic method can thus be recommended for such an analysis and also for the purpose of quality control in the drug industry.

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