

Differential pulse polarographic method development and validation of riboflavin in pharmaceutical formulation

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Abstract- The development of differential pulse Polarographic (DPP) method for determination of riboflavin in pharmaceutical formulation was investigated. As first, we studied the electrochemical behaviour of riboflavin by DPP using a dropping mercury electrode (DME) as working and Hg/Hg₂Cl₂, Cl⁻(sat) as a reference electrode in Britton-Robinson (BR) buffer (pH 3.0–10). The results that were obtained showed that, the BR buffer solution with pH 7.0 was the best medium for reduction of riboflavin on the mercury electrode at peak potential (*E_p*) -1.48 V. The range of linearity was found to be from 1.0 to 16.0 ppm with limit of detection of (LOD) 0.915 ppm and limit of quantification (LOQ) was 3.050 ppm with R² value was 0.997. Statistical analysis proved that the method was precise, reproducible, selective, specific, and accurate for analysis of riboflavin in pharmaceutical formulation.

Keywords - Riboflavin, Differential pulse polarography, Method validation.

I. INTRODUCTION

Riboflavin is very important and essential vitamin for human nutrition, and growth. It plays a key role in biological reduction oxidation process and is available in various foods, but can be found easily in energy drinks, vitamin tablets and other vitamins supplements. These artificial sources are great for people who don't eat certain food that contains these vitamins, or are highly deficient. The deficiency of riboflavin can cause serious damage to our health and appearance.

There are number of electro-chemical methods used by many researchers for the determination of riboflavin includes spectrophotometer [1, 2], photochemical spectrophotometer [3], fluorometry [4, 5], HPLC [6], reverse-phase HPLC [7] and chromatography [8]. Various workers have carried out Polarographic [9], cathodic voltammetric as well as cyclic voltammetry studies of riboflavin [10]. The other specific powerful techniques are also available, which include normal or synchronous fluorescence, radioimmunoassay and enzyme-linked immunosorbent assay which use specific protein-binding selectivities [11]. The microbiological [12] methods of the Association of Official Analytical can also be applied for determination of riboflavin

A revision of the literature has given no evidence about DPP studies related to riboflavin in pharmaceutical formulation. Considering the great advantage of DPP, we have studied electrochemical characterization of riboflavin in pharmaceutical preparation by DPP.

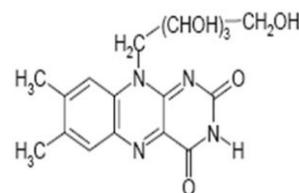


Figure1: Chemical structure of riboflavin

II. MATERIALS AND METHOD

A. Chemicals

The reference standard riboflavin solutions and supporting electrolyte, BR buffer, 0.04 M was prepared in doubly distilled water. The purity of reference standards were 99.9%. All other reagents employed were of analytical grade and used without further purification.

B. Instrumentation

Polarographic analyzer model CL-362 supplied by an Elico Ltd, Hyderabad with PC through its RS 232C interface with the help of ELICO's windows based software were used for polarographic measurements. A dropping mercury as a working electrode, saturated calomel as reference and platinum wire as auxiliary electrodes was used. Spectrophotometric measurement was carried out using UV-VIS spectrophotometer, PerkinElmer Lambda 25, in 1 cm quartz cell. All measurements were made at room temperature.

C. Calibration curve preparation

For DPP studies, a series of nine solutions were prepared in BR buffer pH 7.0 as a supporting electrolyte containing riboflavin concentrations ranging from 1ppm-16 ppm. Each standard and sample solution was transferred in a polarographic cell, degassed with nitrogen by 5 min. and polarograms (**Figure 3**) were recorded from - 3.0 V to -1.6 V at optimized parameter (**Table 1**)

For UV-Visible spectroscopic studies, 15 ppm riboflavin was prepared in 0.04 M BR buffers. The solution was scanned in UV-Visible spectrophotometer in the range 200nm-800nm using 0.04 M BR buffer as a blank. The wavelength corresponding to maximum absorbance (λ_{max}) was found at 444.91 nm. The calibration curve was constructed by taking standard solutions used in DPP (**Figure 6**)

D. DPP assay for tablets

The oral tablet containing 25 mg of riboflavin was purchased from local market and suspended in 25.0 mL distilled water. A 1.0 mL aliquot of each solution was taken and diluted to 50 mL with 0.04M BR buffer solution, pH 7.0. Each sample solution was transferred in a polarographic cell, degassed with nitrogen by 5 min. and recorded at least thrice from -3.0 V to -1.6 V; the concentration of riboflavin in the sample solution were calculated from regression equation prepared from standard calibration curve.

E. UV-Vis spectrophotometry assay for tablets

The oral tablet containing 25 mg of riboflavin was purchased from local market and suspended in 25.0 mL distilled water, sonicated and centrifuged at 3000 rpm. A 1.0 mL aliquot of each solution was taken and diluted to 50 mL with 0.04M BR buffer solution, pH 7.0. Each one sample solution was measured at 444.91 nm, and the concentration of riboflavin in the sample solution was calculated from regression equation prepared for standard calibration curve.

III. METHOD VALIDATION

The DPP method was validated according to international guidelines for bioanalytical methods, including stability of analyte, determination of specificity and selectivity, calibration curve, detection and determination limits, accuracy, and inter-day and intraday precision

A. Linearity and Detection Determination Limits

The linearity of the method was checked by constructing a plot of different concentration of riboflavin versus corresponding peak current (I_p) at peak potential. The solution was scanned from -3.00 V to -1.6 V, for varying the riboflavin concentration ranging from 1.0 ppm to 16.0 ppm in 0.04M BR buffer solution, pH 7.0 (**Figure 4**). The LOD and LOQ for the proposed method were calculated according to the equation $LOD = 3\sigma/m$ and $LOQ = 10\sigma/m$, where σ represents standard deviation of the slope (m) (**Table 1**)

B. Accuracy

To check the degree of accuracy of the method, recovery experiments were performed by tablets assay of riboflavin containing excipients (Dicalcium Phosphate, Microcrystalline Cellulose, Vegetable Stearic Acid, Silica, Vegetable Cellulose, and Vegetable Magnesium Stearate.) according to manufacturer's

batch formulation for 25 mg riboflavin per tablet. Accuracy was assessed as the % recovery (**Table 2**).

C. Precision

Precision study was performed for intra-day and inter-day variation of different solutions of same concentration, were analyzed three times in a three day and the peak current at peak potential is noted. From the I_p mean, standard deviation and %RSD was calculated (**Table 3**). These values were well within the limit of ICH guidelines.

D. Selectivity

The specificity was evaluated by analyzing solutions containing the excipients employed for the preparation of riboflavin in commercial tablets.

IV. RESULTS AND DISCUSSION

A. DPP behaviour of riboflavin

The reversibility of reduction mechanism of riboflavin was investigated at DME by using DPP in BR buffer at different pH in the range of 2-11. Riboflavin produces a well defined peak in a wide range of pH from 2.0 to 8.0. It was observed that sharp and well-defined DPP response was obtained at pH 7.0. Therefore the analytical studies were performed at pH 7.0. At this pH it was found that I_p is directly proportional to the vitamin concentration in the solution. The proposed reduction mechanism at pyridine ring of riboflavin is due to two-electron reduction to generate the dihydroriboflavin derivative in BR buffer (**Figure 2**).

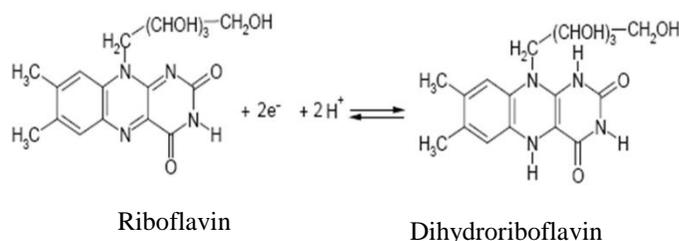


Figure 2: The proposed reduction mechanism of riboflavin

B. Optimization of instrumental conditions

The DPP determination of vitamin at trace level normally involves very small current responses. Therefore optimization of instrumental and experimental parameters has been performed for 10 ppm as highest concentration in BR buffer at optimized pH 7.0.

C. Effect of pulse amplitude and scan rate

The effect of pulse amplitude on the sensitivity of I_p and E_p was checked in the range of 5.0 to 100 mV with optimum conditions. The results showed that the I_p were increased by increasing pulse amplitude to 50 mV, and then leveled off. This is due to the fact, after 50 mV, the peak current broadened. Thus,

50 mV pulse amplitude was selected. Also the influence of scan rate on the I_p and E_p of riboflavin was studied in the range of 3 to 12 mV/s. The scan rate of 6 mV/s would be the best compromise when considering the sensitivity, resolution and speed requirements and was used throughout the analysis of vitamin.

Table.1: Optimized analytical parameters for determination of riboflavin

Instrumental Parameter	DPP	Analytical Parameter	Validation
Peak potential (V)	-1.484	Optimum pH	7.0
Pulse amplitude (mV)	50.0	Concentration range (ppm)	1.0-16.0
Scan rate (mV/sec)	6.0	LOD (ppm)	0.915
Current range (μ A)	10.0	LOQ(ppm)	3.050
Drop time (sec)	1.0	Correlation coefficient (r)	0.997
Scan type	Forward	Slope (ppm)	0.04
CC Compensation (%)	0.00	Intercept (μ A)	0.305
Data acquisition	Slow	Standard deviation	0.09075

D. Validation of the analytical method

The analytical validation study was carried out using optimum parameters to observe a relationship between I_p and concentration of riboflavin. The calibration curve was prepared by a series of standard solution of vitamin. When the concentration of riboflavin was varied, the I_p increased successively. It shows that the range of linearity was found to be from 1.0 ppm to 16 ppm (**Figure 4**).

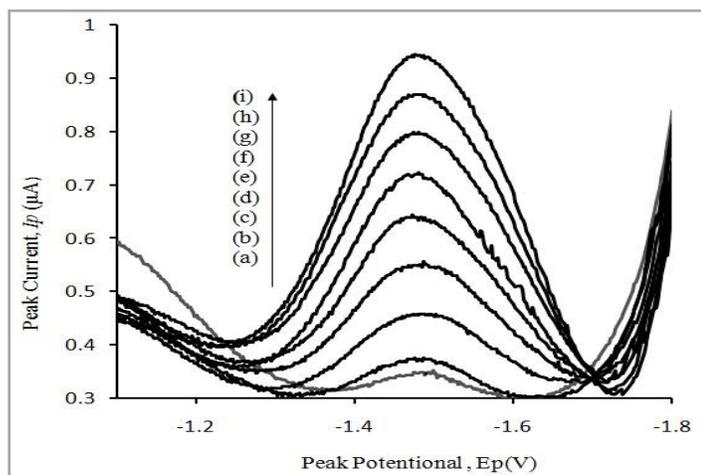


Figure 3: DPP polarogram of riboflavin at pH 7.0 in BR buffer solution obtained at a) 1.0 ppm, b) 2.0 ppm, c) 4.0 ppm, d) 6.0 ppm, e) 8.0 ppm, f) 10.0 ppm, g) 12.0 ppm, h) 14.0 ppm, i) 16.0 ppm.

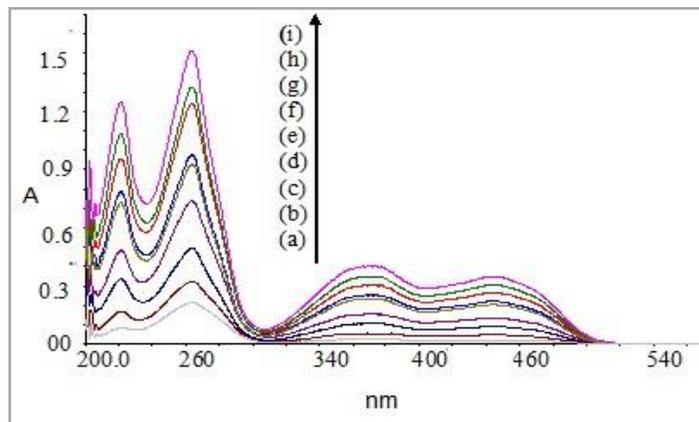


Figure 5: UV/Vis spectrum of riboflavin at pH 7.0 in BRB buffer solution obtained at a) 1.0 ppm, b) 2.0 ppm, c) 4.0 ppm, d) 6.0 ppm, e) 8.0 ppm, f) 10.0 ppm, g) 12.0 ppm, h) 14.0 ppm, i) 16.0 ppm,

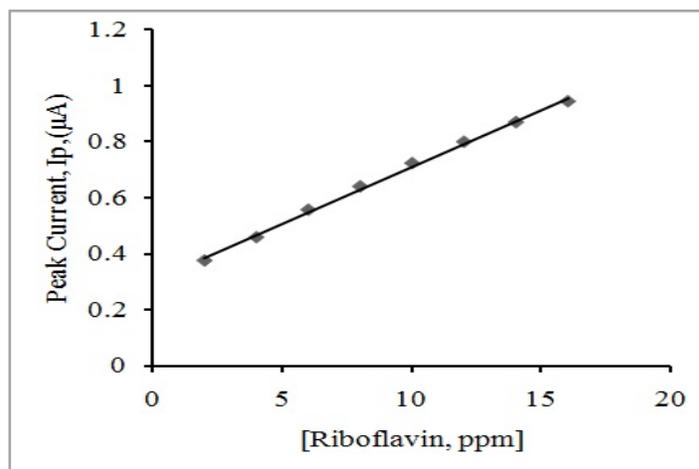


Figure 4: Linear plot of I_p versus concentration of riboflavin at pH 7.0 in BR buffer

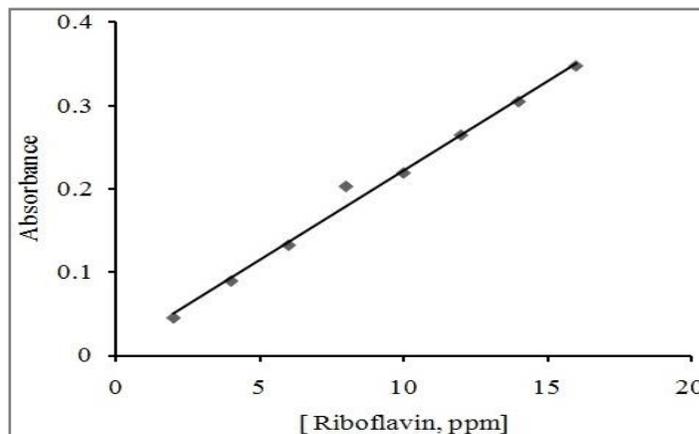


Figure 6: Linear plot of absorbance versus concentration of riboflavin at pH 7.0 in BR buffer

E. Analytical applications

Based on the greater resolution and sensitivity of instruments, DPP and UV technique were applied for quantification in working pH of 7.0. For assay, the calibration plot method was used, obtained from nine points between 1 ppm and 16 ppm expressed by the following equation

$$DPP \quad I_p = 0.04 \times C + 0.305 \quad (R^2 = 0.997, n=9)$$

$$UV \quad A = 0.021 \times C + 0.008 \quad (R^2 = 0.991, n=9)$$

where I_p is the peak current, C is the riboflavin concentrations in ppm, R^2 is the regression coefficient and A is absorbance

Table 2: Recovery of riboflavin from 25 mg per tablet samples by DPP and UV-Vis spectrophotometry

Sample	DPP	UV/Vis
01	98.03	97.36
02	97.23	98.12
03	96.89	98.65
04	97.24	97.89
05	98.26	96.00
06	97.03	98.06
07	96.05	98.89
Average	97.24	97.95
SD	± 0.7465	± 0.9576
%RSD	± 0.7676	± 0.9786

The recovery study in **Table 2** indicates that both DPP and UV-Vis spectrophotometric techniques are adequately precise and accurate with % RSD lower than 1% and percentage of recoveries near 98%. It is recommended for the determination of riboflavin in pharmaceutical formulation.

Table 3: Precision studies by the proposed DPP procedure (n=3)

Riboflavin Vitamin (ppm)	Intraday measurement Ip±SD (%RSD)	Interday measurement Ip±SD (%RSD)		
		1 day	2 day	3 day
4	0.4848±0.0074 (%1.443)	0.4848±0.0074 (%1.443)	0.462±0.0016 (%3.528)	0.4664±0.0061 (%1.300)
8	0.630±0.0066 (%1.046)	0.630±0.0066 (%1.046)	0.6288±0.0082 (%1.30)	0.6121±0.0088 (%1.452)
12	0.7812±0.0092 (%1.180)	0.7812±0.0092 (%1.180)	0.7792±0.0108 (%1.380)	0.7689±0.0120 (%1.560)

F. Conclusion

The proposed DPP method is accurate, precise, reproducible, specific, fast, low cost and stability-indicating methods. The DPP method has a great potential as an alternative method for this application in the future and successfully developed for the determination of riboflavin in pharmaceutical formulations in the presence of other commonly occurring ingredients

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