Adsorptive Stripping Differential Pulse Voltammetry for Determination of Trace Amounts of Riboflavin in Human Plasma

Ali Niazi*
Iran, Arak, Azad University of Arak, Faculty of Sciences, Department of Chemistry
Effat Amjadi
Iran, Arak, Azad University of Arak, Faculty of Sciences, Department of Chemistry

Abstract

A new adsorptive anodic differential pulse stripping voltammetry method for the direct determination of riboflavin at trace levels in human plasma is proposed. The procedure involves an adsorptive accumulation of riboflavin on a hanging mercury drop electrode (HMDE), followed by oxidation of adsorbed riboflavin by voltammetry scan using differential pulse modulation. The optimum conditions for the analysis of riboflavin are pH = 6.5 using borate buffer, accumulation potential of -700 mV (vs. Ag/AgCl), and accumulation time of 70 s. The peak current is proportional to the concentration of riboflavin, and a linear calibration graph is obtained at 0.01-0.90 μgml^{-1}. A relative standard deviation of 0.52% (n = 3) was obtained, and the limit of detection was 6 $ngml^{-1}$. The capability of the method for the analysis of real samples was evaluated by determination of riboflavin in spiked human plasma with satisfactory results.

Keywords: Riboflavin, Adsorptive stripping, differential pulse voltammetry, Human plasma.

Introduction

The vitamins are a diverse group of compounds that they comprise a range of biomolecules whose common properties reside in the fact that they are essential dietary components, which are needed in relatively small amounts to sustain life and good health.[1] The vitamins, depending on their solubility, they are classified in fat-soluble vitamins (Vitamins A, D, E and K) and water-soluble vitamins (B-complex, C, folic acid, pantothetic acid and nicotinic acid). Historically, the B-complex vitamins were measured by microbiological assays: nowadays, there are several more

powerful techniques available, which include high performance liquid chromatography (HPLC), normal or synchronous fluorescence, radioimmunoassay and enzyme-linked immunosorbent assay which use specific protein-binding selectivities.[2]

Several methodologies have been developed for the determination of riboflavin, such as HPLC, spectrophotometry and fluorescence methods[3-6] and several chemometrics methods, such as kalman filtering, principal component regression and partial least squares, were used for determination of vitamins. [3,7,8] However, any methods have been developed for determination of riboflavin using voltammetry. Thus, with the increase in the production and

* Corresponding author

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consumption of drugs that employ riboflavin, it becomes necessary to develop a new method for its determination. Thus, in recent years, stripping voltammetry techniques,[9-17] particularly anodic and cathodic adsorptive stripping voltammetry, have shown numerous advantages, including speed of analysis, good selectively and sensitivity, and low costs of instrumentation compared with other techniques. This method provides low detection limits due to its "built in" preconcentration step. Thus, stripping voltammetry has become a valuable tool for determination of drug such as riboflavin in pharmaceutical formulations and biological fluids. In this work, an adsorptive anodic differential pulse stripping technique was developed for direct determination of the trace measurement of riboflavin in human plasma. The method is based on the effective accumulation of riboflavin (Figure 1) on a hanging mercury drop electrode and oxidation of the adsorbed riboflavin in stripping step. The effect of various parameters including pH, accumulation potential, accumulation time, scan rate and pulse amplitude in the standard solution on the sensitivity and accuracy of method were investigated.

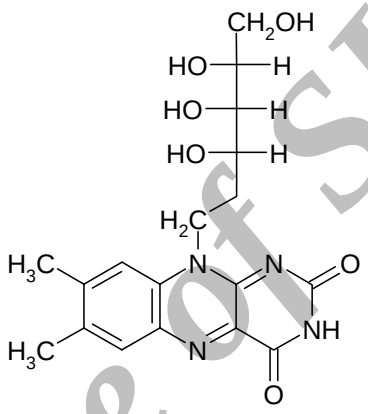

Figure 1. Chemical structure of riboflavin.

Experimental

Reagents and solutions

All chemicals were reagent grade chemicals (Merck or Fluka), doubly distilled water was used in preparation of all solutions. Stock standard solution of riboflavin, 1000 µgml-1, was prepared by dissolving the compound in a minimum amount of NaOH 0.1 M and diluting it with water. This solution was stored in the dark at 4°C and was found to be stable for at least 2 weeks. Borate buffer was prepared by dissolving appropriate amounts of boric acid in water and adjusting to the desired pH value with sodium hydroxide and hydrochloric acid solutions.[18]

Instrumentation

A Metrohm 692 pH-meter furnished with a combined glass-saturated calomel electrode was used to determine pH of the solutions. Voltamograms were obtained by using an Autolab instrument (Eco Chemie, The Netherlands), Model PGSTAT12 processor, with three electrodes consisting of a HMDE (r_0 = 0.20 mm, 0.450 mg) as working electrode, an Ag/AgCl (3.0 M KCl) reference electrode and a carbon counter auxiliary electrode that linked to a computer (Pentium IV, 3.0 GHz). Solutions were deoxygenated with high-purity nitrogen for 2 min prior to each experiment.

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General procedure

All experiments were performed at room temperature. A 50 ml aliquot of borate buffer solution (pH = 6.5) and appropriate volumes of the sample solution were injected into a 50 ml volumetric flask and transferred to the electrochemical cell. The solution was purged with nitrogen first for 3 min and then for 30 sec before each adsorptive stripping step. Then an accumulation potential of -700 mV versus Ag/AgCl was applied to a fresh HMDE during stirring the solution for a period of 70 sec. Following this preconcentration, stirrer was stopped and after equilibrium of 5 sec, the differential pulse voltammogram was recorded from -600 to -200 mV with a potential scan rate of 10 mV s^{-1}. The current was measured and recorded for the sample solution. Peak heights were evaluated as the difference between each voltammogram and the background electrolyte voltammogram.

Analysis of spiked human plasma samples

Plasma spiked with riboflavin solution was obtained by diluting aliquots of the authentic stock standard riboflavin solution with the human plasma. A 20 µl aliquot of this spiked solution was diluted to 1.0 ml with ethanol in 10 ml centrifuge tubes. The precipitated protein was separated by centrifugation for 10 min at 3000 rpm. The clear supernated layer was filtrated through 0.45 µm filter to produce spiked protein free-human plasma of known riboflavin concentration.

Results and discussion

Preliminary investigations

Riboflavin exhibits one oxidation peak close to -400 mV, at pH 6.5 on the HMDE (*vs.* Ag/AgCl / KCl 3 M), as can be observed in differential pulse voltammetry, in Figure 2a, without preconcentration, and after a preconcentration step (-700 mV, 70 s, 2000 rpm) (Figure 2b).[19-21] Comparison of the voltammograms shows that the height of the riboflavin oxidation peak depends on the duration of the preconcentration step.

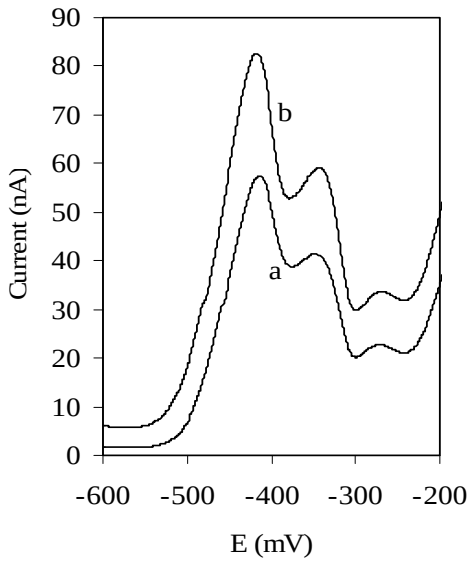

Figure 2**.** Differential pulse voltammograms for riboflavin in borate buffer (pH = 6.5) using a scan rate of 10 mV s^{-1} and pulse height = 80 mV, (a) without accumulation; (b) after a stripping step; accumulation time 70 sec, accumulation potential -700 mV and stirring speed 2000 rpm.

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The peaks obtained without accumulation were substantially smaller than those obtained after a preconcentration step, indicates riboflavin is adsorbed readily at the electrode surface, and a considerable increase in sensitivity can be gained by adsorptive accumulation prior to the voltammetric determination. Taking into account that the differential pulse voltammetry technique was used to develop the method for quantitative determination of riboflavin, and in order to choose the optimum conditions, some analytical and instrumental parameters were studied.

Effect of operational parameters

Taking into account that the differential pulse voltammetry technique was used to develop the method for quantitative determination of riboflavin, and in order to choose the optimum conditions, some analytical and instrumental parameters were studied.

Effect of analytical parameters

Effect of variation of pH and ionic strength

The influence of pH on the stripping peak current for the oxidation peak was examined using differential pulse voltammetry and the results are shown in Figure 3. It can be seen that the peak current reaches a maximum at pH rang 6.0-8.0. Therefore, pH = 6.5 was selected as the optimum pH. At this pH, the sensitivity was highest, the peak was well defined, and the base line was flat.

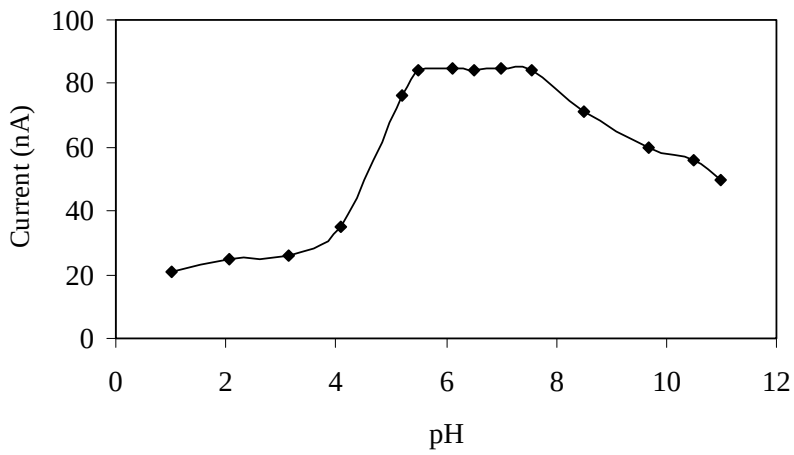

Figure 3. Effect of pH on the peak current. Conditions as in Figure 2.

The effect of ionic strength of the sample solution on the stripping peak current was examined by varying the concentration of $NaNO_3$ up to 1 M. The results showed that the change of ionic strength did not have any effect on peak current response.

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Effect of instrumental parameters

Effect of variation of accumulation potential and accumulation time

The accumulation potential was varied between -1000 and -500 mV during a constant accumulation time of 70 sec. The anodic voltammetric scan was first after an equilibrium time of 5 sec without stirring to allow the electrode to equilibrate before performing the measurement process. Maximum peak current was observed at accumulation potentials of -700 to -400 mV (Figure 4). Generally, an accumulation potential of -700 mV vs. Ag/AgCl was used for further investigation. Variation of the accumulation time between 0 and 150 sec at an accumulation potential -700 mV were investigated.

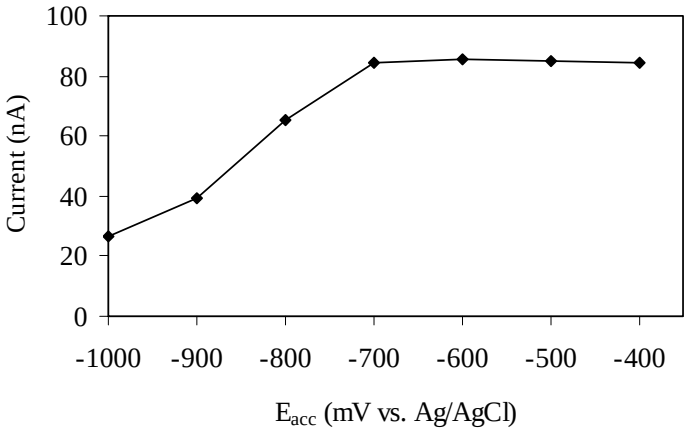

Figure 4. The influence of accumulation potential on the peak current of riboflavin. Conditions as in Figure 2.

Figure 5 shows the dependence of the oxidation peak current on accumulation time for riboflavin. In different concentration, at first, peak current increases with accumulation time, indicating that adsorption equilibrium is taking placed, the longer the accumulation time, the more riboflavin was adsorbed, and the larger was the peak current. However, after a specific period of accumulation time (about 70 sec for high concentration of riboflavin), the peak current leveled off, illustrating that adsorptive equilibrium of riboflavin on the mercury electrode surface was achieved. The long accumulation time leads to an improvement of the detection limit. An accumulation time of 70 sec was selected for riboflavin determination throughout this work as compromise between high sensitivity and short analysis time.

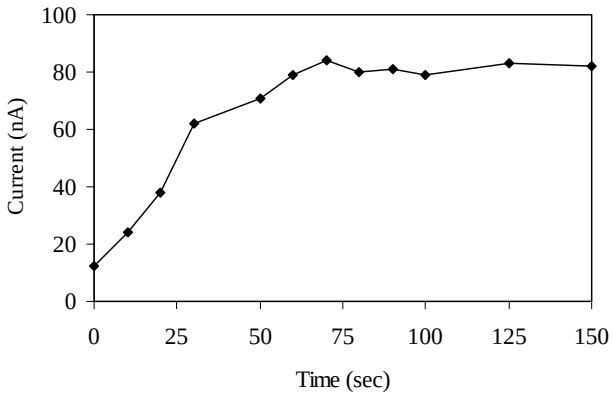

Figure 5. The effect of accumulation time on the peak current of riboflavin. Other conditions as in Figure 2.

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Effect of pulse height and scan rate

The effect of pulse height on the sensitivity of the oxidation current peak was also checked using different pulse heights in the range of 10 and 100 mV with the optimum conditions. The results showed that the peaks current were increased by increasing pulse height to 80 mV, and then leveled off. This is due to the fact, after 80 mV, the peak current broadened. Thus, 80 mV pulse height was selected. The influence of potential scan rate on the peak current of riboflavin was studied in the range of 10-100 mV s^{-1} with the optimum conditions. The results showed that by increasing scan rate from 10 to 40 mV s^{-1}, the peak currents slightly decreased, whereas for higher scan rate the rate of increasing in peaks current decreased. This is due to the fact that at lower scan rate (10 mV s^{-1}) the adsorption processes is the main of phenomena at the electrode surface, whereas at higher scan rate diffusion of the riboflavin from solution to the electrode surface added to the adsorption phenomena.[20] Therefore, a scan rate of 10 mV s^{-1} was selected for study.

Effect of stirring speed, drop size and equilibrium time

The effects of stirring rate, drop size and equilibrium time on the voltammetric response were examined in developing a suitable analytical procedure for the determination of riboflavin. The chosen working conditions were: drop size of 3, stirring rate of 2000 rpm and equilibrium time of 5 sec.

Reproducibility, linear range and detection of the method

The reproducibility of repeating the determination of low concentration of riboflavin was found under the optimum conditions. The RSD for three determinations were calculated as 0.52% for riboflavin concentration of 0.05 µgml^{-1}. Typical calibration graph is shown in Figure 6. Under the optimized conditions the peak current of riboflavin was found to be proportional to its concentration over the range 0.01–0.90 µgml^{-1} and its equation was $[current]_{nA}=2027.1\times[riboflavin]+7.4785$. The correlation coefficient (r) was 0.9992. The detection limit ($Y_{LOD} = \hat{X}_B + 3S_B$), (where Y_{LOD} is the signal for detection limit, $\hat{X}_B$ is the mean of blank signal, and S_B is the standard deviation of blank signal) was obtained as 6 ngml^{-1}.[22]

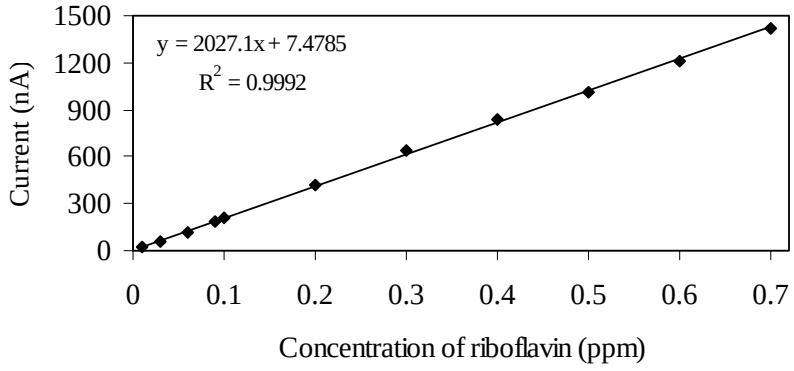

Figure 6. Typical calibration graph for determination of riboflavin in borate buffer (pH = 6.5), accumulation potential = -700 mV, accumulation time = 70 sec, scan rate = 10 mV s^{-1}, pulse height = 80 mV and stirring speed = 2000 rpm.

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Assay of riboflavin in human plasma

The proposed procedure was successfully applied for the assay of riboflavin in human plasma. The results showed that satisfactory recovery for riboflavin could be obtained (Table 1) using the recommended procedure. The data obtained by this method reveal the capability of the method for determination of riboflavin in real samples such as human plasma. The average recoveries with the RSD in determination of riboflavin in human plasma are summarized in Table 1.

Table 1. Determination of riboflavin in spiked human plasma using proposed method compared to actual added (μgml^{-1}).

Samples	Added	Found	RSD	Recovery (%)
Sample 1	0.05	0.047	0.75	94.0
Sample 2	0.25	0.242	0.48	96.8
Sample 3	0.40	0.412	0.43	103.0

Conclusion

The adsorptive differential pulse stripping voltammetry on an HMDE can be used to determine riboflavin at trace levels because of its low detection. It could be determined directly in complex matrices (human plasma) with great success using an anodic stripping differential pulse voltammetry procedure. In conclusion, the above system offers a practical potential for the trace determination of riboflavin, especially with its advantages of acceptable sensitivity and linear dynamic range, simplicity and speed that were not present in the previously reported system.

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