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Short communication

A new proposal for fast determination of vitamin B_2 from aqueous pharmaceutical products

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1. Introduction

The vitamins, between the riboflavin, are an essential group of compounds, which are needed, in small amounts to sustain life and good health. For instance, a protein that inhibits bacterial growth [1] mediates the transport of riboflavin in biological fluids.

Because, the concentrations that are present are usually very low is necessary to use combined methods to detect and analyse them [2].

The current density of an electrode redox reaction occurring with combined overtension of charge transfer and nonstationary linear semi-infinite diffusion of the species Ox, Red is the solution of an integral equation of Volterra type [3].

In the case of linear sweep voltammetry it was possible to transform this integral equation into an ordinary differential equation of first order whose solution describes the experimental voltammograms.

As for the above mentioned differential equation, it has served to develop new methods of direct voltammetry with linear scanning the potential for determining either the kinetic parameters j^0, β of the electrode redox reaction, or the parameters characterising the electrochemical active species in solution, i.e. the diffusion coefficients D_{Ox}, D_{Red}, respective the concentrations c_{Ox}, c_{Red}.

By a new mathematical procedure it has been obtained an expression for $r = c_{Ox}/c_{Red}$ [4]. By using spectral determination and linear sweep voltammetry we have estimated the concentration (c_{Ox}), the quality of vitamine B_2 salt from aqueous pharmaceutical dosage forms.

Finally for redox reversible reactions it is possible to write an invariant depending only on the product $D^{1/2}c_{Ox}$ if the values c_{Ox} and c_{Red} remain in the domain where Nernst equation applies [5].

1.1. The principle of the method

Because the theoretical developments are based on the above-mentioned differential equation we reproduce below the last form of this equation as it

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was recently reported [6]:

$$\frac{1}{A(j^0\bar{N})^2}\left[\frac{dI_F(t)}{dt}-\left[\beta+\frac{1}{\exp(f|v|t)-1}\right]f|v|I_F(t)\right] = \left[1+2\left[\beta-\frac{1}{\frac{\exp(f|v|t)}{r}+1}\right]f|v|t\right] \cdot[\exp(2\beta f|v|t)]\cdot\left[\frac{1}{r}+\exp(-f|v|t)\right] \cdot\left[\left[\frac{1}{r}+\exp(-f|v|t)\right]\frac{\alpha_1(t)I_F(t)}{A} -\frac{\alpha_2(t)[1-\exp(-f|v|t)]}{\pi^{1/2}\bar{N}t^{1/2}}\right] \quad (1)$$

where

$$\bar{N}=1/F\sqrt{D_{Red}}c_{Red};\qquad r=\sqrt{\frac{D_{Ox}}{D_{Red}}}\frac{c_{Ox}}{c_{Red}};$$

$$f=F/RT$$

$|v|$ is the magnitude of the scanning rate, $I_F(t)$ the intensity of the cathodic Faradaic current, A the electrode area, D the diffusion coefficient and c_{Ox}, c_{Red} the concentration of electrochemical species. As for the functions $\alpha_1(t)$, $\alpha_2(t)$ it was not possible to give explicit forms of them, but it has been shown [6] that they must satisfy the condition:

$$\frac{\alpha_2}{\alpha_1}(t)=\alpha_2(t)/\alpha_1(t)\to 1 \quad \text{for great values of t}$$

The method supposes the presence of an indifferent electrolyte in excess and consequently, the double layer capacity C_d is round 20 μF/cm^2. Then, using scanning rates no greater than 100 mV/s, it follows that the density of capacity current is very small, i.e. $i_c=|v|C_d<2\times10^{-6}$ A/cm^2, and $I_F(t)$ may be considered equal with the current intensity I(t) as measured in the external circuit.

In this paper we shall consider only the cathodic voltammograms because we refer to the case when the oxidised species Ox is the one having therapeutical properties. The other case, when the species Red is the medicine will be considered in a future paper and then the anodic voltammograms will be necessary.

The exchange current density j^0 is related to the concentrations c_{Ox}, c_{Red} by the well-known equation [7]:

$$j^0=j^{00}c_{Ox}^{1-\beta}c_{Red}^{\beta} \quad (2)$$

where j^{00} represents the standard exchange current density. Using Eq. (2) and the expressions of $\bar{N}$ and r, it follows:

$$(j^0\bar{N})^2=\left(\frac{j^{00}}{F\sqrt{D}}\right)^2 r^{2(1-\beta)} \quad (3)$$

where it has been taken into account that generally, $D_{Ox}\cong D_{Red}=D$.

For irreversible reactions, $j^{00}\to 0$ (i.e. the charge transfer occurs with maximum difficulty) and thus $(j^0\bar{N})^2\to 0$ for finite values of r. Consequently, the square bracket of the first member of the Eq. (1) must cancel irrespective of the value of t (because only in this way the first member of Eq. (1) gets the indeterminate form 0/0).

For reversible reactions, $j^{00}\to\infty$ (i.e. the charge transfer occurs with any difficulty) and thus $(j^0\bar{N})^2\to\infty$. Consequently, the first member of Eq. (1) is equal to zero irrespective of the value of t, conclusion that applies to the second member too. The only possibility is the cancellation of the last square bracket of the second member irrespective of the value of t, i.e. of $|\eta|=|v|t$. It thus results:

$$\frac{|\eta|^{1/2}I(|\eta|)}{\exp(f|\eta|)-1} =\frac{A|v|^{1/2}F\sqrt{D}c_{Ox}}{\pi^{1/2}}\frac{\alpha_2}{\alpha_1}(|\eta|)\frac{1}{\exp(f|\eta|)+r} \quad (4)$$

Further, one must observe from Eq. (3) that if $r\to\infty$, $(j^0\bar{N})^2$tends to infinity even for quasireversible reactions (when j^{00} has a finite value). Consequently, the method may be applied in the general case of quasireversible reactions if r has a sufficiently great value. However, this is just the case which we are interested in, because only the oxidised species having therapeutical properties, the concentration c_{Red} must be reduced as much as possible in the sold medicine.

Coming back to Eq. (4), for two overtensions $|\eta_i|$ and $|\eta_i+1|$, the first before, the second after the normal cathodic peak, which appear at $|\eta_p|$ satisfying the condition:

$$I(|\eta_i|) = I(|\eta_{i+1}|) \tag{5}$$

(for small distances $|\eta_i+1|-|\eta_i| = \Delta_i$ as compared with the overtensions$|\eta_i|$) and accepting the approximation:

$$\frac{\alpha_2}{\alpha_1}(|\eta_i|)/\frac{\alpha_2}{\alpha_1}(|\eta_{i+1}|) \cong 1 \tag{6}$$

we can write:

$$\frac{|\eta_i|^{1/2}}{|\eta_{i+1}|^{1/2}} \cdot \frac{e^{f|\eta_{i+1}|}-1}{e^{f|\eta_i|}-1} = \frac{e^{f|\eta_{i+1}|}+r}{e^{f|\eta_i|}+r} \tag{7}$$

$$\frac{1}{\left(1+\frac{\Delta_i}{|\eta_i|}\right)^{1/2}} \cdot \frac{e^{f|\eta_i|}-e^{-f\Delta_i}}{e^{f|\eta_i|}-1} = \frac{e^{f|\eta_i|}+re^{-f\Delta_i}}{e^{f|\eta_i|}+r} \tag{7a}$$

and for very small Δ_i

$$\left(1-\frac{1}{2}\cdot\frac{\Delta_i}{|\eta_i|}\right)\cdot\left(1+\frac{f\Delta_i}{e^{f|\eta_i|}-1}\right) = 1-\frac{fr\Delta_i}{e^{f|\eta_i|}+r} \tag{8}$$

Neglecting in the first member the term in Δ_i^2, one gets:

$$-\frac{1}{2f|\eta_i|}+\frac{1}{e^{f|\eta_i|}-1} = -\frac{r}{e^{f|\eta_i|}+r} \tag{9}$$

equation, which of course, is valid also for $|\eta_i| \to |\eta_p|$. It thus results:

$$r = \frac{1+2x_p-e^{x_p}}{1-2x_p-e^{-x_p}} \tag{10}$$

where we have denoted $x_p = f|\eta_p|$, value that results from the cathodic voltammogram, corresponding to the cathodic peak η_p.Introducing Eq. (10) in Eq. (4) and performing algebra (for $\eta = \eta_p$) one finally gets:

$$\frac{x_p^{1/2}I_p|v|^{-1/2}}{1-\frac{1}{x_p}+\frac{1}{2x_pe^{(x_p)}}} = A\cdot\frac{Ff^{1/2}D_{Ox}^{1/2}c_{Ox}}{\pi^{1/2}} \tag{11}$$

The function

$$\Phi(x_p,\ I_p,\ |v|) = \frac{x_p^{1/2}I_p|v|^{-1/2}}{1-\frac{1}{2x_p}+\frac{1}{2x_pe^{(x_p)}}} \tag{12}$$

must be an invariant with respect to the changing of |v|, whatever the value of r would be; of course the values of concentrations remain in the domain where Nernst equation applies.

From Eqs. (11) and (12)

$$D_{Ox}^{1/2} = \frac{\pi^{1/2}\phi(x_p,\ I_p,\ |v|)}{AFf^{1/2}c_{Ox}} \tag{13}$$

By spectrophotometric method it is possible to determine the c_{Ox} value, and from electrochemical data the value of r and ϕ. The two combines methods gets finally the diffusion coefficient.

2. Experimental

A Unicam Helios β spectrophotometer equipped with 1 cm quartz cell and internal software was used to collect the spectral measurements. The following instrumental parameters were used: λ range 300–600 nm and scan speed 400 nm/s.

The working standard solution was prepared by addition of corresponding amounts of B_2 vitamin 5′-phosphate mono-sodium salt (Merck) in 0.01 M NaCl solution to realise stock solution of 10^{-4} M.

Direct voltammograms were performed using a BAS 100 potentiostat. Control of the potentiostat and data acquisition was accomplished using BAS 100 electrochemistry software. All the electrochemically measurements were made in a three-electrode cell, using glassy carbon electrode as a working electrode supplied by BAS Inc. The area of working electrode was $A = 7.10^{-6}$ m^2. As reference electrode we used an Ag/AgCl electrode and a Pt wire as a counter electrode. Between each measurement the working electrodes were polished with diamond paste and washed with deionised water. The analysed sample was prepared from pharmaceutical dosage containing B_2 vitamin salt (0.014 g), and citric acid (0.002 ml) in 2 ml distilled water. All solutions were deoxygenated by bubbling pure nitrogen for up to 10 min. All chemicals

were used as received and were of analytical grade (Merck). All solutions were made with deionised water prepared using Millipore water, provided from a Milli-Q Lab apparatus.

3. Results and discussions

Riboflavin salts is a yellow compound having two peaks in visible domain at 371 and 445 nm.

The spectral characteristics of vitamin were determined in presence of an indifferent electrolyte in excess at room temperature (Fig. 1). The concentration range of B_2 vitamin salt was 0.6×10^{-4} up to 0.8×10^{-4} M.

In Fig. 2 is presented the calibration curve with its usual parameters (Fig. 2).

Before electroreduction, interpolation of sample in the linear range is in accordance with the expected data ($c_{Ox} = 0.75 \times 10^{-4}$ M).

We are used this concentration as the initial value for the voltammetry with linear scanning potential. Direct first cathodic voltammogram in this case is plotted in Fig. 3.

A first observation mark out the great value for $r = 26.59 \times 10^6$ obtained by calculus using expression (10). In consequence the pharmaceutical probe is of high purity in oxidised specie, i.e. one may neglect the concentration c_{Red} existing in solution.

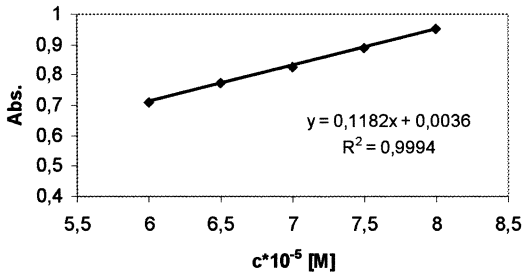

Fig. 2. The calibration curve for the determination of the B_2 vitamin salt concentration from pharmaceutical dosage by spectrophotometrical way at $\lambda = 445$ nm.

The B_2 vitamin undergoes redox reaction during biochemical processes. The redox reaction simulated under electrochemical condition involve a drift of peak potential to lower values due to the appearance of the reduced species. In fact by cycling the probe it was possible to modify the r parameter and the change in the r value has been essential. Thus after 50 cycles the peak potential is about −280 mV per Ag/AgCl (Fig. 4) (in compar-

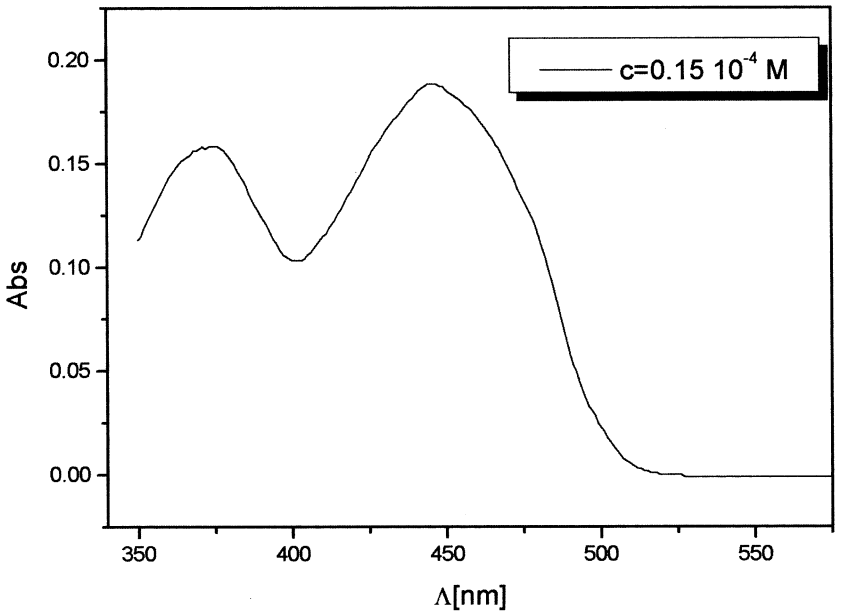

Fig. 1. The UV–VIS spectrum of the B_2 vitamin salt.

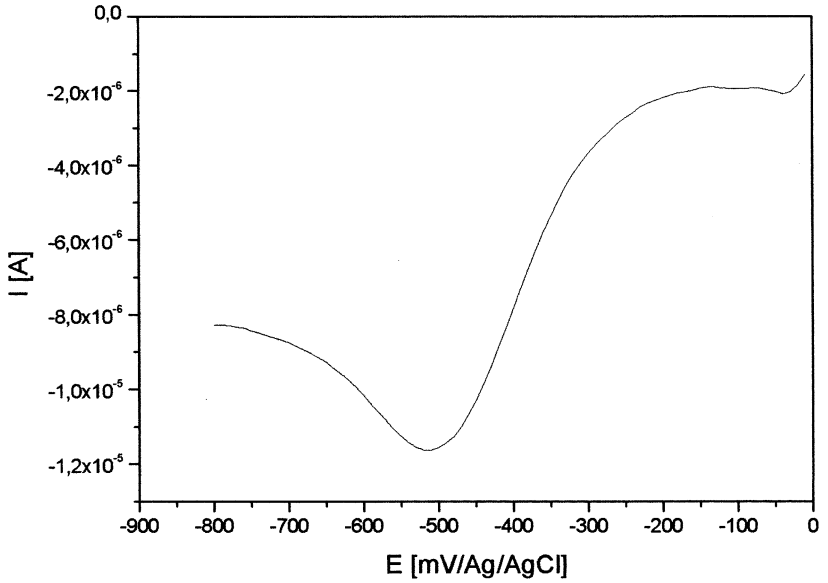

Fig. 3. Direct voltammogram plotted after first scan on glassy carbon electrode at $c_{Ox} = 0.75 \times 10^{-4}$ M.

ison with peak position after the first scanning at −520 mV per Ag/AgCl) and the value of r become r = 3417. In Table 1 are also given intermediate values of r, $|\eta_p|$ and corresponding current intensities I_p, obtained during this cycling process of the probe. The dependence log I_p versus log r has proved to be linear and the equation of the regression line is given in the last column. As for

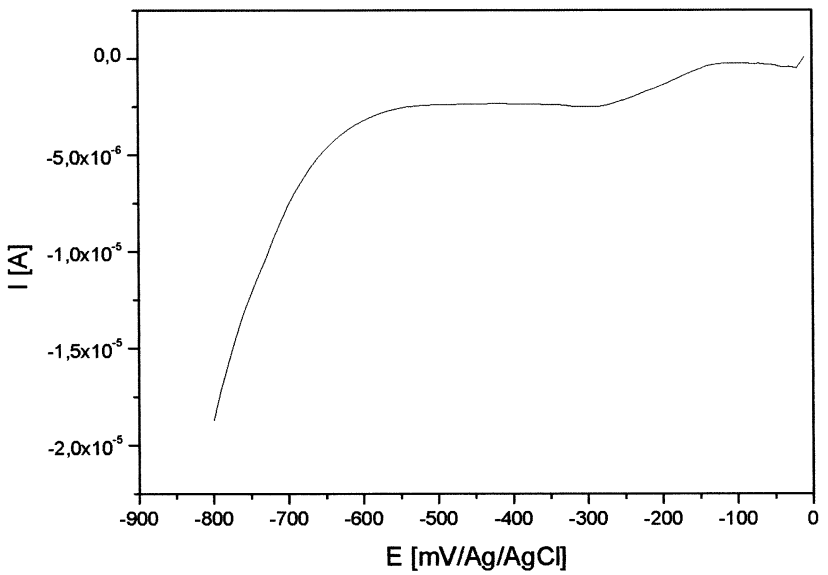

Fig. 4. Direct voltammogram plotted after 50 scans on glassy carbon electrode at $c_{Ox} = 0.68 \times 10^{-4}$ M.

Table 1
The equation of regression line $\log I_p$ vs. $\log r$ resulted from the experimental voltammograms obtained by cycling the probe

v (V/s)	$\lvert\eta_p\rvert$ (V)	$x_p = f(\eta_p)$	$y = \log r$	$z = \log I_p$	Regression line
0.05	0.520	20.8	7.425	−4.936	$z = -6.19 + 0.167y$, $R^2 = 0.91$
0.05	0.360	14.4	4.810	−5.381	
0.05	0.310	12.4	4.001	−5.561	
0.05	0.290	11.6	3.692	−5.582	
0.05	0.280	11.2	3.533	−5.607	

Table 2
Estimation of the diffusion coefficients

r	x_p	$y = \log r$	$z = \log I_p$	$10^6 I_p$ (A)	$10^6\Phi$ (x_p, $I_p\lvert v\rvert$) ($A/V^{1/2}\ s^{1/2}$)	c_{Ox} (mol/m^3)	10^9 D (m^2/s)	$10^9\ \bar{D}$ (m^2/s)
1	2.18	0	−6.19	0.65	5.57	0.038	3.86	5.97
10	4.50	1	−6.02	0.95	10.10	0.068	4.04	
100	7.21	2	−5.86	1.38	17.80	0.075	10.00	

spectral determination on the product resulted after 50 cycles, it also shows a change of c_{Ox} to 0.68×10^{-4} M.

By using the equation of the regression line given in Table 1 it was possible to get the values of I_p corresponding to the values 1, 10 and 100, of r. This extrapolation has permitted to enter in the domain of concentrations where the Nernst equation holds true, which represents a necessary condition for getting valid estimates of the diffusion coefficients $D = D_{Ox} \cong D_{Red}$ by mean of Eq. (13). The values of x_p have been obtained by using Eq. (10), of I_p by means of the regression line equation given in Table 1, and of Φ (x_p, I_p $|v|$) by Eq. (12). The c_{Ox} values have resulted from the initial values 0.075 mol/m^3 (spectrophotometrically determined) and corresponding values of r. Because the probe has proved to be pure, the values corresponding to $r = 100$ is $c_{Ox}98/99 \approx c_{Ox} = 0.075$ mol/m^3. The values resulted for D is given in Table 2.

This procedure has the great advantage of entering in the domain of value r where the formula deduced for getting the value of D applies without significant errors, by using only the test solution.

4. Conclusions

In the present paper, we propose a fast and easy combined method to control the quality of pharmaceutical injectible dosage of riboflavin 5′-phosphate mono-sodium salt.

The possibility of estimating the ratio r is very important in all analytical determination, involving biological fluids. In the same time, the value of r is crucial for estimating the assimilation capacity of the corresponding biological active compound.

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