

VOLTAMMETRIC DETERMINATION OF MELATONIN AND PYRIDOXINE (VITAMIN B6) IN TABLETS

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ABSTRACT

Melatonin and Vitamin B6 can be oxidized at the glassy carbon electrode showing well-resolved anodic peaks by differential pulse voltammetry. Vitamin B6 shows an oxidation peak potential of about 650 mV vs Ag/AgCl in ammonia buffer pH 9 and the peak of melatonin shows an peak potential of 840 mV vs Ag/AgCl in Britton-Robinson buffer pH 2. Both peaks show a linear relation with concentration and consequently are useful for quantification.

Key Words: Melatonin, Pyridoxine, Voltametric determination, Vitamin B₆.

RESUMEN

Melatonina y Vitamina B6 puede ser oxidada en el electrodo de carbono vitreo produciendo picos anódicos bien resueltos mediante Voltametría de Pulso diferencial. La Vitamina B6 revela un pico de oxidación con un potencial de pico de 650 mV vs Ag/AgCl en tampón de amoniacó pH 9, mientras que melatonina produce un pico con un potencial de pico de 840 mV vs Ag/AgCl en el tampón Britton-Robinson pH 2. Ambos picos muestran una relación lineal entre la corriente de pico y la concentración que es utilizada para la cuantificación.

Palabras Claves: Melatonina, Piridoxina, Determinación voltamétrica, Vitamina B6

INTRODUCTION

The hormone melatonin (N-acetyl-5-methoxytryptamine) ([Figure 1](#)) is of considerable interest due to its modulating role of a series of physiologic processes, like the circadian rhythms (1) and neuro-endocrine processes. It also appears to have important antioxidant property (2).

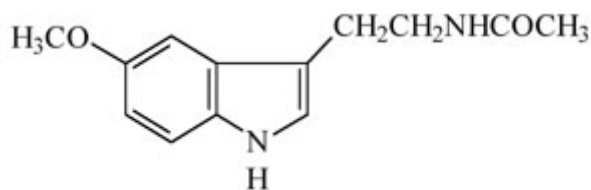


Fig.1.Chemical structure of melatonin.

In recent years melatonin has been found to have a number of direct and indirect roles in protecting cells and organisms from oxidative damage produced by toxic radicals, generated from reactive oxygen species (3). The main application of melatonin is in the treatment of jet-lag, sleep disturbances and cancer (4). Generally melatonin is formulated in combination with vitamins. A popular product marketed in USA is the combination of melatonin with vitamin B6.

There are several methods described for the analytical determinations of melatonin such as high performance liquid chromatography (HPLC) (5,6), radioimmunoassay and fluorometric methods (7,8,9). Recently a voltammetric determination in capsules was published (10).

Pyridoxine (Vitamin B6) have been determined by HPLC with fluorometric and electrochemical detection (11,12) and spectrophotometry (13).

The simultaneous analysis of pyridoxine and melatonin in tablet formulation by derivative ultraviolet spectroscopy was recently developed (14), but a revision of the literature gives no evidence about voltammetric determination of melatonin and vitamin B6 in the same tablet. In this work we describe an easy analytical methodology to determine selectively melatonin and vitamin B6 combined in tablets. The determination involves the oxidation of both compounds by differential pulse voltammetry at a glassy carbon electrode. Both substances are oxidized, and to obtain the necessary selectivity two separate runs in different supporting electrolytes were needed.

EXPERIMENTAL

Reagents and drugs

Melatonin, pure drug, was obtained from Farmacias Ahumada S.A. (Santiago, Chile). Vitamin B6, pure drug, was obtained from Merck. Melatonin tablets, Melatonin Schiff tablets, (declared amount melatonin 3.0 mg + 10 mg vitamin B6, Salt Lake City, U.T. U.S.A.) were obtained commercially. All other reagents employed were of analytical grade and were used without further purification.

Standard and working solutions

Stock solutions of 1×10^{-3} M melatonin and vitamin B6 were prepared in EtOH. The working solutions were prepared by dilution in supporting electrolyte. For recovery studies a mixture of melatonin and vitamin B6 plus excipients was prepared according to manufacturer's batch formulas, simulating a tablet composition,

Supporting electrolyte

For melatonin determination a solution of pH 2.0 Britton -Robinson buffer (mixture of 0.04 M boric, acetic and phosphoric acid) was used. For the vitamin B6 measurement the supporting electrolyte was 0.25 M ammonia buffer pH 9.0 (NH₃-NH₄Cl).

Tablet assay

Ten series of one tablet is dissolved with sonication in 50 mL ethanol. A 5 ml aliquot is diluted to 50 ml with pH 2.0 B-R buffer for melatonin determination (theoretical concentration 2.58×10^{-5} M). Another aliquot of 0.750 ml was diluted with pH 9 ammonia buffer for vitamin B6 measurement (theoretical concentration 1.46×10^{-5} M). The applied potential scan was between 0.6 and 1.1 V for melatonin and between 0.4 and 0.8 V for vitamin B6.

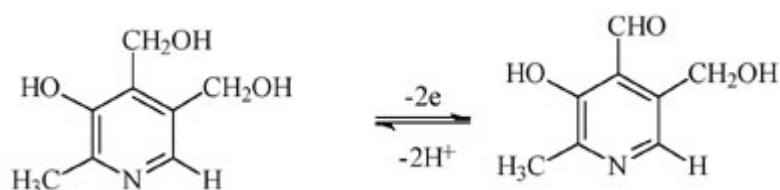
Apparatus

The voltammetric measurements were carried out with a Metrohm equipment as described previously (15). A conventional three-electrode cell system consisting of an Ag/AgCl/KCl 3M reference electrode, a platinum wire counter electrode and a glassy carbon electrode (2mm diameter) as working electrode. Before using this electrode it was necessary to activate its surface. The activation procedure of the electrode was the following: washing with distilled water, putting the electrode in fresh supporting electrolyte solution and applying 250 potential scans at 25 V/s between 0.2 and -1.8 V for the melatonin measurement and between 2 and -1 V for the vitamin determination. This treatment was necessary, in order to obtain better reproducibility of the measurements.

RESULTS AND DISCUSSION

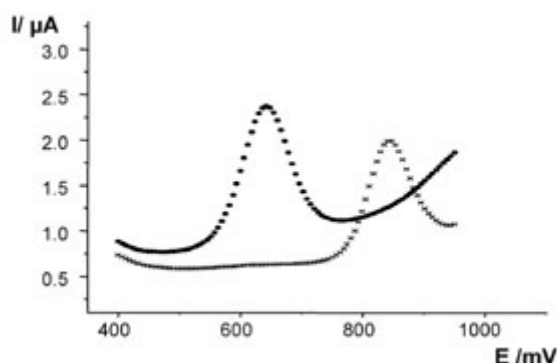
Melatonin and vitamin B6 are formulated in the same tablet and consequently the determination of one in the presence of the other is a real necessity. From the voltammetric point of view this determination is not possible in the same run because melatonin produces a well resolved oxidation peak in acidic media and vitamin B6 in alkaline media. Consequently, we try with a novel procedure that involves two different runs changing the supporting electrolyte.

The oxidation of Vitamin B6 and related compounds in ammonia buffer is well-known and the determination of vitamin B6 in pharmaceutical forms has been published (16). The overall oxidation process is described by the following equation:



The oxidation peak potential is about 650 mV vs Ag/AgCl in ammonia buffer pH 9. Furthermore, we have found, that the voltammogram of vitamin B6 was completely unaffected by the presence of melatonin. On the other hand melatonin produces a well resolved differential pulse voltammogram in all the pH range in BR buffer. The dependence of the peak potential (E_p) with pH is shown in figure 2a. The dependence of the peak current with pH is showed in figure 2b. According to these behavior we have selected pH 2 for quantitative determination. At this pH a peak potential of 840 mV vs Ag/AgCl was observed. In the selected media the voltammetric response of melatonin was totally unaltered by the presence of vitamin B6.

Fig.2.



Differential pulse voltammetric peaks for melatonin in pH 2.0 Britton-Robinson buffer and pyridoxine in pH 9.0 ammonia buffer. Both substances are in mixture containing 6.9×10^{-5} M each. • = vitamin B6, x = melatonin. GCE, potential sweep rate 10 mV/s, Pulse amplitude 50 mV, pulse duration 40 ms.

According to previous work (17) the oxidation process for melatonin is described as follows:

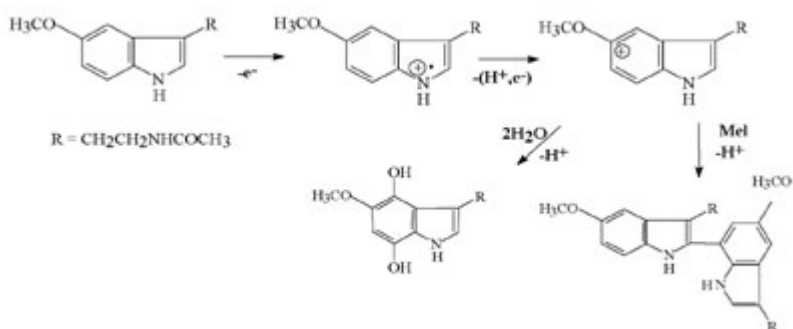


Figure 3 shows the differential pulse voltammetric response in both media for a mixture of melatonin and pyridoxin, both 6.9×10^{-5} M.

Concentration studies

Calibration curves were made with both substances present in a mixture of the same concentration. A linear calibration graph was obtained in the range 5×10^{-6} to 5×10^{-5} M both for melatonin and pyridoxine using differential pulse voltammetry (DPV). This range could be extended, but some loss of linearity is observed due to adsorption of melatonin on the working electrode.

The following regression lines are obtained:

Melatonin:	I_p (μA)	$46573.8 C_1(M) -$ 0.07211	$(r =$ $0.99811)$
Vitamin:	I_p (μA)	$19369.9 C_2(M) -$	$(r =$

$$= 0.0208 \quad (0.99989)$$

where, I_p is the peak current in μA and C_1 and C_2 are the molar concentrations of melatonin and vitamin B6 respectively, r is the regression coefficient for $n=10$.

The reproducibility of the measurements was calculated from ten independent runs of 1×10^{-5} M mixed melatonin and vitamin solutions. The results expressed as the coefficient of variation are C.V.= 0.63% for melatonin and C.V.=1.73% for vitamin. The detection limits were 4.9×10^{-7} M for melatonin and 1.85×10^{-7} for vitamin B6. On the other hand the quantification limit were 8.6×10^{-7} M, and 3.0×10^{-7} M for melatonin and vitamin B6, respectively.

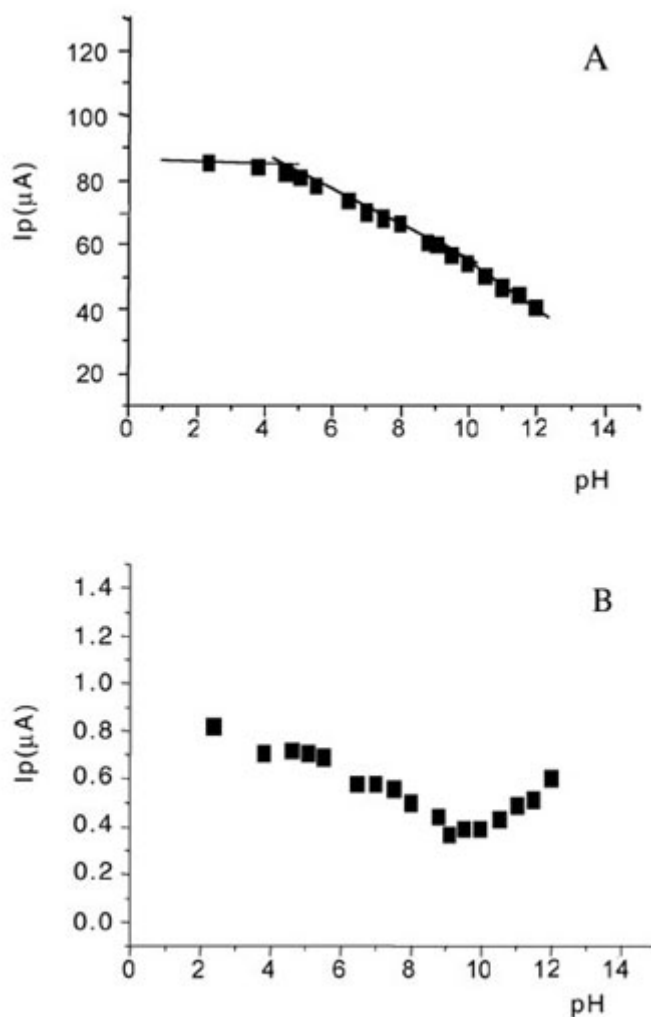


Fig.3. a) Potential peak evolution with pH, b) peak current evolution with pH. (5×10^{-4} M melatonin solution, differential pulse voltammetry on GCE, potential sweep rate 10 mV/s, Pulse amplitude 50 mV, pulse duration 40 ms)

Accuracy of determination

This determination was made by the standard addition method for samples with unknown matrix composition(18). The experimental t student value of the recovery percentage was obtained. This value must be lower than the t -table value of 4.303 ($p=0,05$; $G_i= 3-1=2$).

\bar{X} = Mean value of the

$$t_{\text{exp}} = \frac{|100 - \bar{X}| \sqrt{n}}{C.V.}$$

recovery
 n= Number of additions.
 C.V= Coefficient of
 variation

We obtained values of 1.659 for melatonin and 1.31 for vitamin B6 showing a good accuracy for the proposed method.

Recovery

This study show a recovery of 105 and 107% for melatonin at $7 \times 10^{-6} \text{M}$ and $3 \times 10^{-5} \text{M}$ concentration levels respectively, for $n=10$. For vitamin B6 these values are 95.7 and 99.8% recovery at the same concentrations ($n=10$).

Tablet content uniformity

The developed method was applied to determine melatonin and vitamine B6 in commercial tablets. The individual assay of 10 tablets are given in [table I](#).

Table I. Individual tablet assay of Melatonin and vitamin

Tablet	Individual Tablet Assay	
	Melatonin, mg/tablet	Vitamin B6, mg/tablet
1	2.71	9.78
2	3.10	10.10
3	3.10	10.14
4	2.84	10.10
5	2.97	9.68
6	3.39	10.16
7	3.39	9.70
8	3.05	9.88
9	2.95	10.19
10	3.15	10.06
Average	3.06	9.98
Sd	0.22	0.20
C.V.(%)	7.05	1.99

The USP XXIII pharmacopoeia states (19) that the tablet content of active principle must be between 85 and 115% of the declared amount. Consequently, all the obtained values are within the established range in pharmacopoeia for manufactured products (19).

Based on the above results, we conclude that the developed method is a good alternative tool to be applied for determining both melatonin and vitamin B6 in the same tablet. The method shows good repeatability, adequate reproducibility and recovery. Furthermore, the analytical procedure is easy and the excipients do not interfere in the analysis.

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