Artigo

Cost-effective Composite Electrode for the Fast Voltammetric Screening and Determination of Riboflavin (B2) and Pyridoxine (B6) in Pharmaceuticals

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Eletrodo Compósito de Baixo Custo para o Rápido *Screening* Voltamétrico e Determinação de Riboflavina (B2) e Piridoxina (B6) em Produtos Farmacêuticos

Resumo: Diferentes compósitos sólidos por dispersões mecânicas de partículas de grafite em parafina aquecida (65-80% de grafite, em massa) foram preparados e avaliados visando suas aplicações em procedimentos eletroquímicos e eletroanalíticos de bioanálise. Tais compósitos foram, ainda, avaliados por meio de técnicas termoanalíticas, com o objetivo de estudar a sua conservação e estabilidade a longo prazo (mais de oito meses sem um cuidado especial). Os melhores resultados foram encontrados em 80% de grafite m/m em parafina. Tais eletrodos combinam baixo custo, estabilidade, sensibilidade, facilidade de manutenção e limpeza, possibilidades de fabricação de diversas formas e formatos (com ou sem modificações) e aplicabilidade numa vasta gama de pH. Estudos eletroquímicos usando diferentes técnicas voltamétricas que envolvem vitaminas do complexo B (riboflavina e piridoxina) levaram a um melhor entendimento sobre seus processos eletro-oxidativos sobre eletrodos compósitos de carbono, especialmente sobre a reversibilidade e dependência do pH. Dados foram também obtidos e otimizado com fins analíticos, sendo voltametria a onda quadrada em pH 4,2 escolhido por suas inúmeras vantagens. Boa linearidade entre as respostas de picos, como função da concentração, foi alcançada (5 - 43 μmol L^{-1} de riboflavina, pico em -0,257 V; e até 8,5 x 10^{-4} mol L^{-1} de piridoxina, pico em + 1,04 V) empregando melhores condições estudadas; limites de detecção para ambos os analitos mostraram ser cerca de 1.0 μmol L^{-1}. Diferentes amostras comerciais foram analisadas para riboflavina (EMS ® xarope do complexo B) e piridoxina (Citoneurin 5000 Merck ® ampolas) proporcionando 96,6% e 98,7% de recuperação, respectivamente.

Palavras-chave: Análise voltamétrica; riboflavina; piridoxina.

Abstracts

Different solid composites made by mechanical dispersions of graphite particles into heated paraffin (from 65 to 80% graphite, in mass) were prepared and assessed in order to optimize their use in electrochemical and electroanalytical procedures for bioanalysis. Besides these, composites were also evaluated by thermoanalytical techniques aiming to study their conservation and long-term stability (over eight months without special care), among others. Best results were found at 80% m/m graphite in paraffin. Such electrode combines low-cost, stability, sensitivity, ease of maintenance and clearance, besides the possibilities of manufacture in many different forms and shapes (with or without modifications) and applicability in a wide range of pH. Electrochemical studies by different voltammetric techniques involving vitamins from complex B (riboflavin and pyridoxine) leaded to a better understanding about their electrooxidative processes onto carbon-composite electrodes, specially regarding reversibility and pH-dependence. Data were also acquired and optimized with analytical purposes, being square-wave voltammetry in pH 4.2 chosen by its many advantages. Good linearity between peak responses as function of concentration were reached from 5 to 43 μmol L^{-1} for riboflavin (peak at -0.257 V) and up to 8.5 x 10^{-4} mol L^{-1} for pyridoxine (peak at +1.04 V), best studied conditions; limits of detection (at an S/N of 3) for both analites showed to be circa 1.0 μmol L^{-1}. Different commercial samples were analyzed for riboflavin (EMS$^{®}$ complex B syrup) and pyridoxine (Citoneurin 5000 Merck$^{®}$ ampoules) providing 96.6% and 98.7% recoveries, respectively.

Keywords: Composite electrodes; voltammetric analysis; riboflavin; pyridoxine.

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Cost-effective Composite Electrode for the Fast Voltammetric Screening and Determination of Riboflavin (B2) and Pyridoxine (B6) in Pharmaceuticals

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1. Introduction

Composites are hybrid materials that, despite their homogeneous aspects at macroscopic scales, have well-defined boundaries at microscopic scales. In electrochemistry, composite electrodes consist of mixtures between, at least two phases, one insulating and one conductor. [1,2] Since their first description by Adams,[2] such electrodes are usually made by one liquid phase (e.g. organic solvents,[2] mineral oils,[3] silicone,[4] polyurethanes with or without chemical modifications,[5-23] and others) in which conducting particles (carbon derivatives such as graphite,[3,24-27] nanotubes,[3,25,28-29] graphene sheets,[3,25,29] glassy carbon particles[3,25,29] or even doped diamond [3,25]) are dispersed, the proportion between phases will determine not only the conductivity of the sensor but also the mechanical properties (such as hardness) of the electrode.

This kind of electrode allows lots of applications in many different media, in different shapes and sizes; this can also be easily modified in surface or in bulk, improving sensibility and selectivity, without loss of reproducibility of electroactive area. As previously cited, among the possibilities for the conducting phase, the use of carbon derivatives are widely used, specially due to their relatively good conductivity, stability, resistance in a large range of potential and low cost.[5,9,24,30,31]

Vitamins are essential components in human dietary, the unbalance such nutrient is prejudicial for the organism, what turns their control very important. Between more common and important vitamins of the B complex, riboflavin (B_2), thiamine (B_3) and the pyridoxine (B_6) take special place.[32,33]

In this context, many efforts aiming to develop new strategies to determine these vitamins are identified, often such analysis have being described by using chromatography, or even different kind of sensors as in.[32,34-37] Despite the many advantages of electroanalysis, electrochemical techniques are not commonly used, being found few references, mainly if compared with other kinds of analysis, and even though, it is used to be seen the use of robust sensors as in.[38-40] The use of electrode composites after chemical modifications is also possible as presented by.[31]

Despite the fact that both paraffin and carbon (in its graphite form) are very stable and low-cost, these substances are not very used as composite for unmodified electrodes. Recently literature shows an interesting citation regarding solid wax composite electrode applied to the determination of ethambutol in pharmaceuticals by flow analysis systems.[41]

This article intends to describe some uses for the graphite-paraffin electrode composite applied in the determination of complex B vitamins (vitamin B_2, riboflavin, and vitamin B_6, pyridoxine) in aqueous media by voltammetric techniques.

2. Experimental

2.1. Reagents and solutions

All reagents and salts were of analytical grade of purity and used as received, without further purification or treatment steps; solutions of electrolytes and vitamins standards were prepared just before use by direct dissolution of respective solutes in bi-distilled deionized water, amber volumetric flask were chosen aiming to avoid light-exposure effects. Riboflavin and pyridoxine hydrochloride standards were obtained at local market, being produced by Pharmanostra®, Italy.

Different buffer solutions (pH from 1.2 to 12.0) were prepared, being such solutions used not only for characterizing the electrooxidation processes for both vitamins under studies but also to define best conditions for the fast analytical

determination in real samples.

Two different commercial samples were purchased and analyzed in the present work, the first consisted of a complex B multivitamin syrup produced by EMS®, the second sample consisted of parenteral use ampoules produced by Merck®; both samples were produced and commercialized in Brazil.

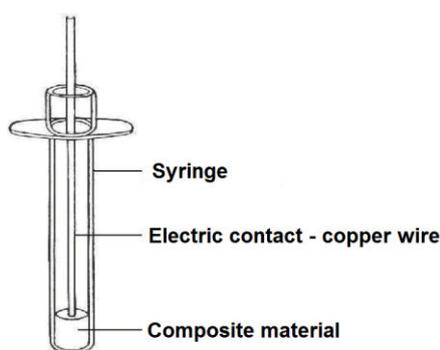## 2.2. Composite electrodes preparation

Composite electrodes were prepared by mixing different proportions of solid paraffin (Solven Wax 170/190®, from Solven®, Brazil) and graphite (particles diameter < 20 μm, from Sigma-Aldrich®, United States of America). These materials were mixed and melted at 60°C until the obtainment of a homogeneous composite material, the initially aimed proportions were 65, 70, 75 and 80% (m/m graphite in paraffin). The composites reached were then compressed and sealed inside plastic syringes, being the electric contact made by metallic wires, as illustrated in Figure 1.

Figure 1. Scheme for the electrode set up

The working electrode consisted of different proportions between graphite particles and paraffin (from 65 to 80% m/m) solid dispersion, prepared as previously described in Perantoni *et al.*[41] In brief, suitable amounts of paraffin and graphite were mixed in small glassy cups keeping constant masses to reach every assessed proportion graphite/insulator; this mixture was then heated at 60°C and inserted in a suitable plastic syringe, providing, this way, rods with 0.5 cm length. Copper wires were applied to obtain electrical contact. After suitable polishment using paper sheets a 0.125 cm^2 geometrical surface area was reached; more detailed procedure can be found above.

2.3. Apparatus

Thermogravimetric data (TGA/DTG and DTA) curves were recorded using a SDT-Q600 simultaneous TGA-DTA thermal analyzer controlled by Thermal Advantage (4.2.1) software, from TA Instruments, under a 100 mL min^{-1} nitrogen or air flow (different atmospheres for different expected steps in the same analysis), samples consisted of ca. 10 mg of each evaluated material (from pure paraffin to every prepared composite) suitable placed into Al_2O_3 pans being temperature swept from room temperature to 900°C at a 10°C min^{-1} heating rate (β).

For electrochemical measurements a conventional three-electrode cell (50.0 mL capacity) consisting of a graphite-paraffin composite working electrode (in different

assessed proportions), a platinum wire counter-electrode and Ag|AgCl reference was used. This system was coupled to an Ivium CompactStat® potentiostat (Ivium Technologies, The Netherlands) controlled by IviumSoft® software (also from Ivium Technologies), being all measurements carried out at room temperature (26 ± 1°C).

3. Procedures

3.1. Electroanalytical determination and evaluation of electroactive areas

The electroactive surface areas were determined according to [13,16,25] by cyclic voltammetry, using 5 mmols L^{-1} hexacyanoferrate (II) (used as an outer-sphere redox system probe) in 0.5 mol L^{-1} KCl (supporting electrolyte) at different scan rates (from 10 to 100 mV s^{-1}), sweeping from -0.2 to +0.8 V vs Ag|AgCl reference electrode and applying the Equation 1:

$$I_{pa}(A) = 2.95x10^5 n^{3/2} A\sqrt{D}C_{\infty}\sqrt{v} \qquad (1)$$

Where *n* is the number of electrons involved in the oxidation, *A* is the electroactive area (cm^2), *D* is the diffusion coefficient of the probe used in such electrolyte (7.7 x 10^{-6} cm^2 s^{-1})[13], C_{∞} the bulk concentration of the probe, and *v* the potential scan rate, in V s^{-1}.

3.2. Thermoanalytical characterization of the electrodes

Aiming to evaluate possible chemical and/or physical interactions between graphite and paraffin, as well as to assess the stability of the used composites all over the time, some thermoanalytical experiments were carried out by both thermogravimetry (TG) and differential thermal analysis (DTA).

Experiments were done using the samples of pure paraffin (Solven Wax 170/190®) and of different composites obtained (65-80% m/m graphite/paraffin), these were weighted (~12.0 mg each) and analyzed in alumina crucibles, from room temperature (~25 °C) to 700°C at 10°C min^{-1} heating rate under dynamic N_2 atmosphere (100 mL min^{-1}), after this temperature, the atmosphere was changed to synthetic air at the same flow rate until 900 °C.

3.3. Voltammetric procedures

Different electroanalytical procedures were carried out under many different conditions such as pH value for supporting electrolyte, potential scan rates and intervals, among others. Cyclic voltammetry was optimized by applying different scan rates (10, 25, 50, 75 and 100 mV s^{-1}), from -1.0 to +1.4 V (*vs* Ag|AgCl reference electrode), square-wave voltammetry was also optimized by combining different parameters such as frequency (10, 25, 50 and 100 Hz), amplitude (10, 25 and 100 mV) and step potential (5, 10 and 25 mV).

The electrochemical behavior of riboflavin was evaluated in different values of pH by using square-wave voltammetry, best conditions for both sensitivity and resolution were 25 mV step potential, 50 mV amplitude and 100 Hz frequency; sweeping from -1.0 to +1.2 V, differences in response between blank and spiked solutions (2.8 x 10^{-5} mol L^{-1} RIB) were measured.

Besides this, the electrochemical behavior of pyridoxine was also investigated by cyclic voltammetry in different values of pH, sweeping from 0.0 to +1.4 V at a scan rate of 25 mV s^{-1}, Three sequential cycles were recorded for each assessed value of pH and the differences in response between blank and spiked solutions (5.0 x 10^{-4} mol L^{-1} PIR) were measured.

For analytical purposes, best conditions for a fast square-wave voltammetric quantification were reached and cited before, in the case of pyridoxine determination the unique difference is that better peak profiles were obtained by changing the frequency from 100 Hz to 50 Hz; in all cases best peak profiles were found at pH 4.2 acetate buffer.

Before each experiment a step of pre-treatment of the electrode was done, consisting of five cycles from -0.8 to +1.4 V at 100 mV s^{-1}. This step not only could clean the electrode surface but also activate carbon particles.

3.4. Commercial samples descriptions and preparation

Different commercial samples were purchased and assessed, the first was a complex B multivitamin syrup produced by EMS$^®$, the second sample consisted of ampoules produced by Merck$^®$, their complete compositions are presented in Tables 1 and 2.

Table 1. Complete composition for Complex B multivitamin syrup from EMS®

Complex B multivitam from EMS$^®$	
Component	**Amount of respective solute for each mL of sample**
Calcium pantotenate	1.5 mg
Cianocobalamin	1.5 mcg
Nicotinamide	2.5 mg
Pyridoxine hydrochloride	1 mg
Riboflavin	1 mg
Thiamine hydrochloride	3 mg

Table 2. Complete composition for ampoule of Citoneurin 5000$^®$ parenteral solution, from Merck®

Citoneurin 5000 ampoules from Merck$^®$	
Component	**Amount of respective solute for each mL of sample**
Pyridoxine hydrochloride	100 mg
Thiamine hydrochloride	100 mg

Such samples were submitted to analytical determination by square-wave voltammetry under the optimized conditions for each analite, as previously described. Riboflavin contents were determined in syrup samples from EMS$^®$ without any treatment, aliquots of 250 μL from such sample were directly added 50.0 mL supporting electrolyte pH 4.2 being followed by standard addition of successive aliquots from an aqueous stock solution ([RIB] = 1 mmol L^{-1} and [PIR] = 0.02 mol L^{-1}). In the case of ampoule samples from Merck$^®$ it was necessary to carry out a previous dilution (10 μL of the sample to 50.0 mL pure water) and, from the diluted sample aliquots of 1.0 mL were taken, further analytical steps followed the same way above described for the first sample.

4. Results and discussion

4.1. Electroactive areas

Figure 2 presents a comparison between results found by the determination of

electroactive surface areas for the different composite electrodes in triplicate and their expected value according to geometric areas. As expected, proportions between 65% and 75% showed no significant difference, which can be explained by the random distribution of graphite particles in graphite matrix in association to the fact that the final conductivity is the non-linear sum of conductive contributions from graphite to

insulate properties from paraffin, proportions lower than 65% showed no suitable conductivity for a sensor as well as contents higher than 80% in graphite presented bad mechanical properties, being friable and difficult to seal. By plotting peak current *vs* scan rate the electroactive area was found to be circa 0.105 cm^2, corresponding to 84% of geometric area (0.125 cm^2).
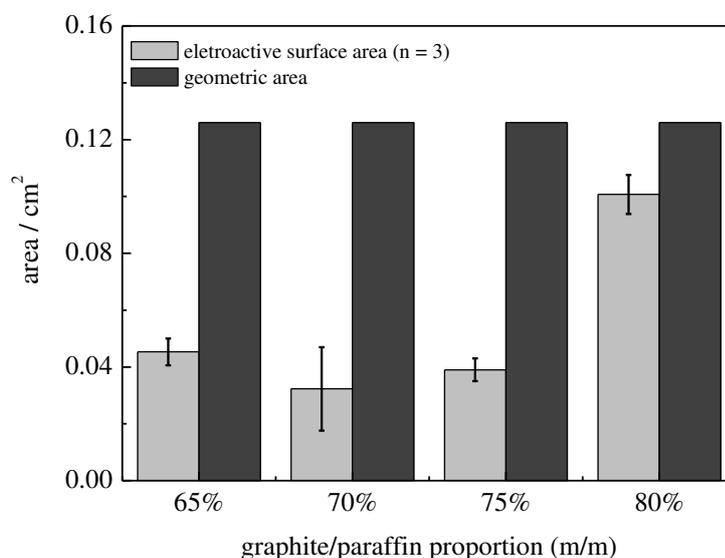

Figure 2. Comparisons between geometric and electroactive surface areas found for the different prepared electrodes

4.2. Thermoanalytical studies

Samples were assessed by termogravimetry, by heating under nitrogen dynamic atmosphere up to 700°C, and thus the atmosphere was changed to air, letting, this way, carbon to burn (900°C); detailed

conditions were previously presented. Thermogravimetric data were used in order to determine the exact graphite content in each composite after eight months storage (see Figure 3). Typical curves for pure paraffin and the prepared composites showed, as expected, no variation over the storage time, even no special care was taken.
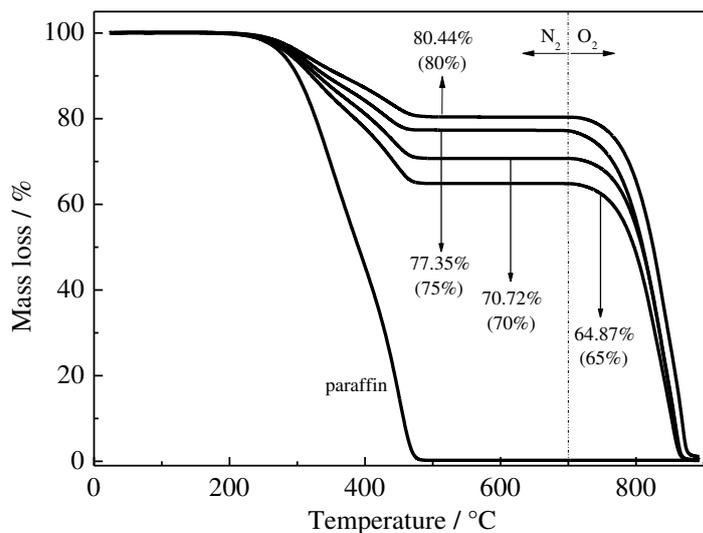
Figure 3. TG curves for the pure paraffin and different prepared composites under previously described conditions

The pure paraffin presents thermal stability until close 220°C, after this, the complete decomposition occurs in successive overlapping steps between 220 °C and 500 °C (being a great amount of energy delivered at 497°C) without producing residue in the sample holder. Besides this, the melting point (66.8°C) of such insulator was evaluated by differential thermal analysis (DTA), being in agreement to the temperature used for composite preparation (Figure 4).
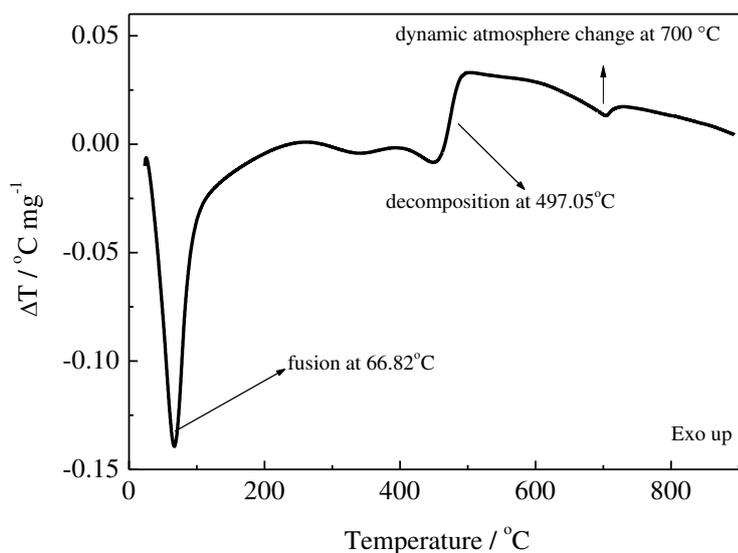
Figure 4. Simultaneous DTA curve obtained for the pure paraffin under previously described conditions

The composites presented a mass loss correspondent to that observed in pure paraffin at the same temperature range. An intense carbon burn can be observed close to 700°C, when the furnace atmosphere is changed to synthetic air. Thermogravimetric results permitted to determine the graphite content in the composites, after changing the furnace atmosphere from nitrogen to air occurred a decomposition step related a mass loss due to the carbon content. The results demonstrated that homogeneous and stable materials can be obtained using the simple preparation procedure described.

### 4.3. Influence of pH and mechanistic studies for riboflavin and pyridoxine

Aiming to asses de relationship between pH and electrochemical responses, cyclic and square-wave voltammetry were carried out by applying the optimized conditions to a series of buffers from 1.2 to 12.0, analyzing the differences between blanks and spiked solutions prepared as previously described. Such experiments allowed a better understanding of possible electrooxidative mechanisms for both riboflavin and pyridoxine as well as provided the most suitable value o pH for the electroanalytical measurements.

Since a reversible electrooxidation path is expected for riboflavin, its mechanism was evaluated by square-wave voltammetry (amp = 50 mV, step potential = 25 mV, f = 100 Hz), subtracting peak profiles reached (2.76 µmol L^{-1} spiked solutions) in each value of pH from respective blanks, Figure 5(a) shows the relationship between pH and peak potential with slope close to 59 mV, which suggests the involvement of the same number of protons and electrons in a reversible process (demonstrated by respective cyclic voltammetry profiles).

In the case of pyridoxine, which undergoes to an irreversible process, cyclic voltammetry was carried out, being peak profiles subtracting from their respective blanks (sweeping at 25 mV s^{-1}, pH from 1.3 to 13.0, spiked solutions containing 5×10^{-4} mol L^{-1}), in addition Figure 5(b) shows the relationship between pH and peak potential with slope close to 55 mV, which suggests the involvement of the same number of protons and electrons.

Possible electrooxidative mechanisms for riboflavin and pyridoxine, in agreement with those presented by [23, 32, 42] are illustrated in Figure 6.
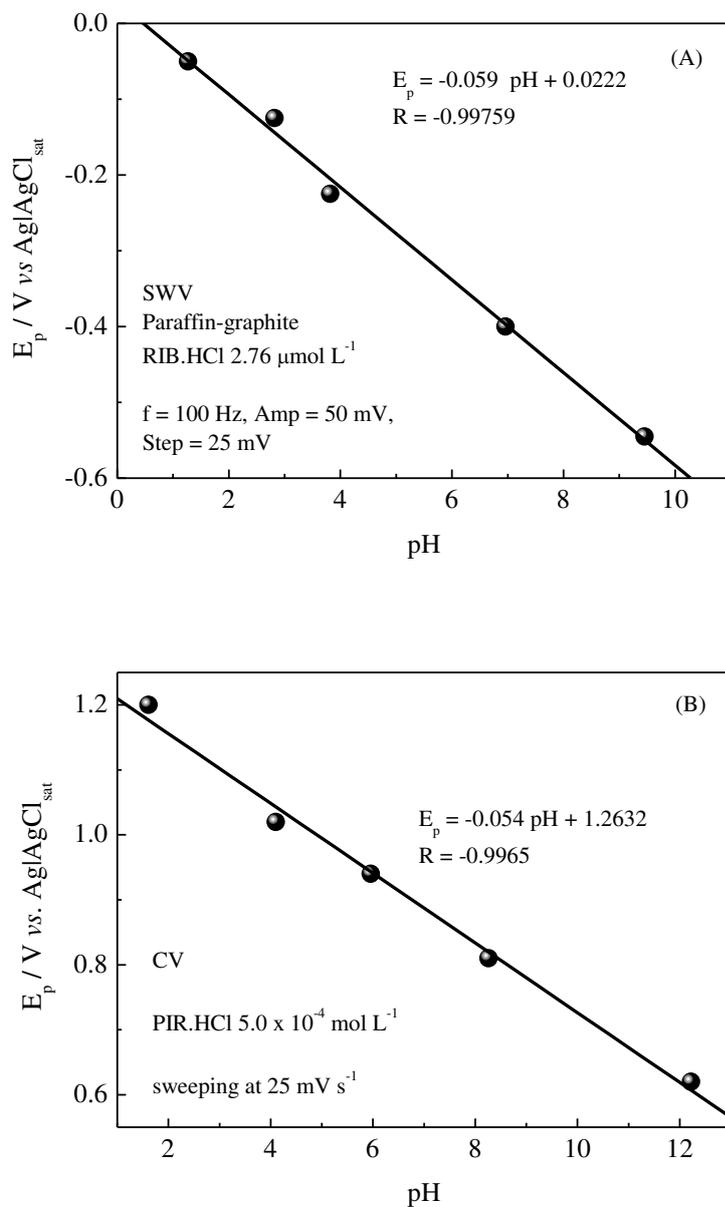
Figure 5. Dependence of the: (a) square-wave voltammetric peak potential on pH for 2.8 µmol L^{-1} riboflavin spiked solutions; (b) cyclic voltammetric peak potential on pH for 5 x 10^{-4} mol L^{-1} riboflavin spiked solutions
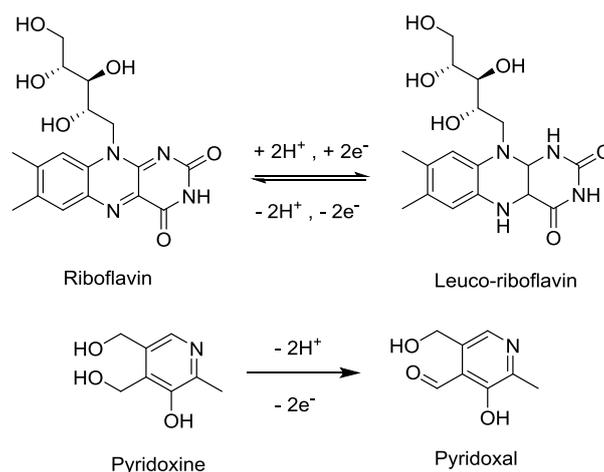
Figure 6. Possible electrooxidative processes for riboflavin and pyridoxine

4.4. Electroanalytical procedures and sample analysis

Samples were purchased at local market and prepared as described in section 2.5. Experiments using square-wave voltammetry were carried out in order to determine riboflavin and pyridoxine in the different samples cited, for this pH 4.2 acetate buffer was used as supporting electrolyte.

For the analysis of riboflavin in syrup samples best conditions showed to be 100 Hz frequency and 25 mV potential increment, with 50 mV amplitude; analytical signals (oxidation peaks) at -0.257 V were used in order to dete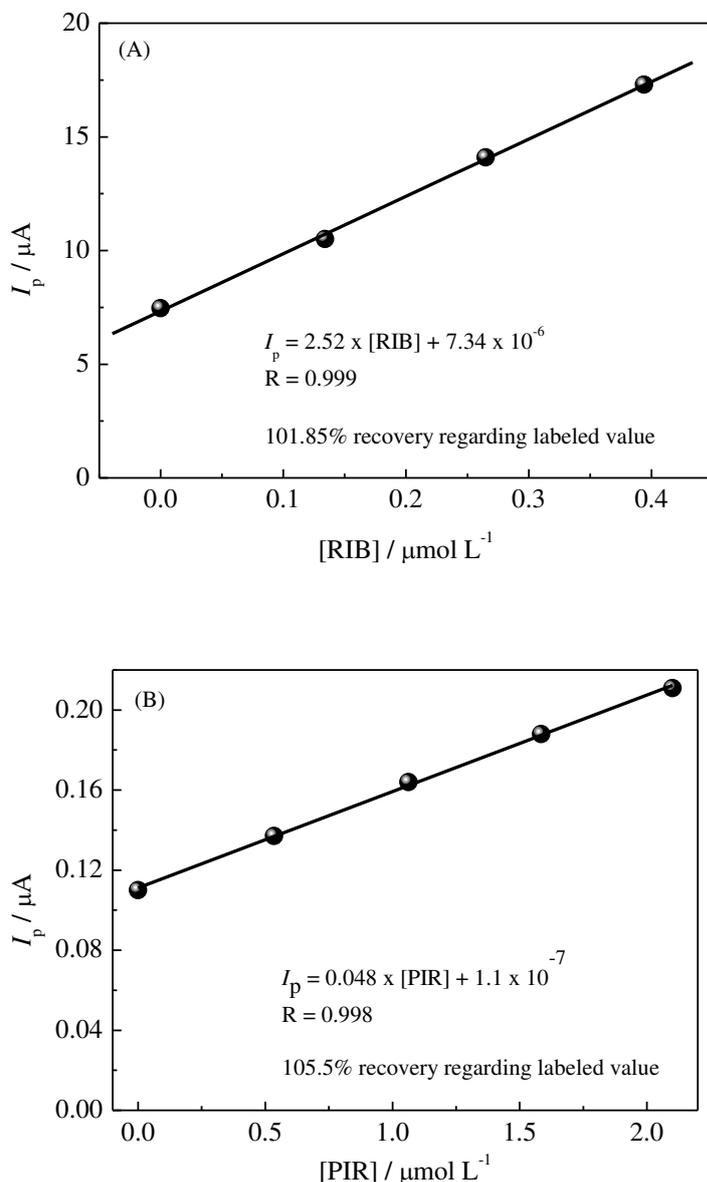rmine such analite by standard addition procedure. On the other hand, aiming to determine pyridoxine in ampoule samples, frequency must be changed to 50 Hz, being analytical signals detected at +1.04 V. Figure 7 presents analytical profiles while sweeping from -0.8 to +1.4 V under the conditions cited for riboflavin analysis, in such case the linear range for riboflavin seemed to be from 5 to 43 μmol L^{-1}, while pyridoxine's linear region showed to be up to 8.5 x 10^{-4} mol L^{-1}; despite this large linear region pyridoxine analysis showed to be viable only at lower concentrations, probably due to saturation on the double-layer (presence of many other components from real samples). Limits of detection for both analites showed to be circa 9.8 x 10^{-7} mol L^{-1}.
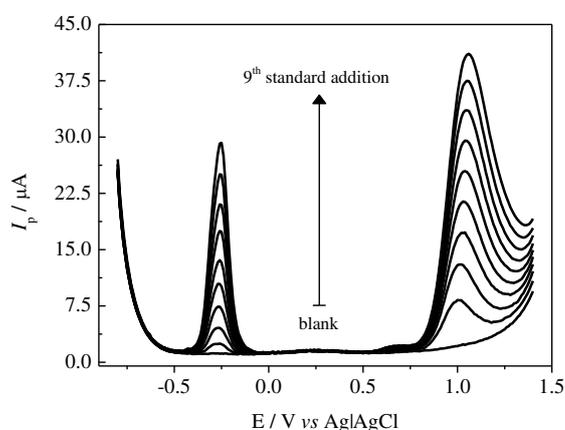
Figure 7. Square-wave voltammograms for successive additions of 250 μL stock solution ([RIB] = 1 mmol L^{-1} and [PIR] = 0.02 mol L^{-1}) into 50.0 mL pH 4.2 acetate buffer supporting electrolyte under optimized conditions for riboflavin determination

Careful examination of standard addition curves for both analysis (see Figure 8 (a) and (b)) suggests their applicability to fast screening and determination of the analites in such samples. For riboflavin determination recoveries were found to be (96.6 ± 8.0)% while for pyridoxine it showed to be (98.7 ± 6.8)%, in both cases analysis were done in authentic triplicates.

(A)

$I_p = 2.52 \times [RIB] + 7.34 \times 10^{-6}$
R = 0.999

101.85% recovery regarding labeled value

(B)

$I_p = 0.048 \times [PIR] + 1.1 \times 10^{-7}$
R = 0.998

105.5% recovery regarding labeled value

Figure 8. Square-wave voltammetric determination by standard addition under the optimized and previously described conditions for: (a) riboflavin in syrup; (b) pyridoxine in ampoules

5. Conclusions

Considering many advantages of composite electrodes such as costs, stability, sensitivity, ease of maintenance and clearance, possibilities of preparation in many different forms and shapes (with or without modifications) and applicability in a wide range of pH, different solid composites

made by mechanical dispersions of graphite into heated (~65°C) paraffin were prepared and assessed in order to optimize their use in electrochemical and electroanalytical procedures. Such materials were also evaluated by thermoanalytical techniques aiming to study their conservation, showing a long-term stability over eight months, without special care of maintenance or storage. As presented and discussed best results were found at 80% m/m graphite in paraffin. Electrochemical studies by different voltammetric techniques were carried out for riboflavin and pyridoxine in different values of pH, providing confirmations and a better understanding about their electrooxidative processes onto carbon-composite electrodes, specially regarding reversibility and pH-dependence. From the analytical point of view square-wave voltammetry in pH 4.2 was chosen due to its many advantages; good linearity between peak responses and concentration were reached from 5 to 43 μmol L^{-1} for riboflavin and up to 9 x 10^{-4} mol L^{-1} for pyridoxine, being the peak potentials sufficiently resolved; limits of detection for both analites showed to be circa 1.0 μmol L^{-1}. Different commercial samples were analyzed for riboflavin (EMS$^{®}$ complex B syrup) and pyridoxine (Citoneurin 5000 Merck$^{®}$ ampoules) providing 96.6% and 98.7% recoveries, respectively. The proposed material seemed to be a good and reliable alternative, even for commercial purposes due to its many assessed characteristics.

Acknowledgements

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