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Development of a reversed FIA system for the spectrophotometric determination of Sb(III) and total Sb in antileishmanial drugs

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Abstract

This paper reports the development of a reversed flow injection system for the spectrophotometric determination of Sb(III) and total Sb in antileishmanial drugs. The analytical system is based on the selective reaction between Sb(III) and bromopyrogallol red (BPR) with the decrease of the absorbance at 555 nm. Total Sb concentration was determined after reduction of all Sb(V) to Sb(III) with KI and ascorbic acid. The influence of system variables (chemical and flow type) and the possible interference of high amounts of Sb(V) on Sb(III) was studied as well as the suitable conditions for preparation of samples. It was verified that the use of Triton X-100 enhanced the sensitivity of the methodology and that the previous sonication of the samples was fundamental to achieve accurate results. Under optimized conditions the reversed FIA system was able to process 63 samples per hour with a detection limit of 29 ng ml^{-1} and a R.S.D. of 3.8% (0.25 μg ml^{-1} level). Real samples of commercial antileishmanial drugs were analyzed, being observed no statistical difference between the results obtained by the developed system and FAAS or manual methodology in relation to total Sb concentration.

Keywords: Antimony; Antileishmanial drugs; Flow injection analysis; Spectrophotometry

1. Introduction

The leishmaniasis is an infectious disease disseminated all over the world with remarkable incidence in developing countries of Asia, Africa and Central/South Americas. It can attack both humans and some animal species, being firstly related by Pedro Pizarro in 1571. In Brazil, the leishmaniasis is found along poorest regions of the country and around 67,000 new cases were diagnosticated in 2003, making leishmaniasis to be classified as an out-of-control disease [1].

Although new drugs are in development [2–5], the treatment of leishmaniasis is usually carried out with antimony-based preparations. Among all antimony-based drugs utilized, maybe the most importants are those prepared with *N*-methylglucamine antimoniate, or simply meglumine antimoniate. In this compound Sb is found in the pentavalent form, which is less toxic when compared with trivalent one, which has great affinity by blood and spleen, being also observed its accumulation in the liver and kidney [6]. This way, the determination of the oxidation state in which Sb is found in the medicine is very important to assess the possible deleterious effect of the drug supplied to leishmaniasis patients.

The determination of Sb in different kinds of samples has been performed by various analytical techniques such as IC-ICP-MS [7], hydride generation-atomic fluorescence [8,9], HG-ICP OES [10–12], spectrophotometry [13–17], HG-AAS [18] and HG-FTIR [19]. Among these works only some of them are applied to the Sb determination in antileishmanial drugs [15–19]. Additionally, very few papers relate the speciation of antimony in such samples [16–18].

Gallignani et al. [19] developed a FIA system for HG-FTIR determination of total antimony in pharmaceuticals. The methodology is based on the on-line treatment of the samples in order to release Sb from organic molecules, and posterior reduction of Sb(V) to Sb(III). From Sb(III), gaseous SbH_3 is formed, which is then transported by a N_2 carrier stream to the flow-cell, after separation from solution in a gas–liquid separator device. The 1893 cm^{-1} band was used for quantification

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purposes, providing a detection limit of 0.9 mg l^{-1}, which is adequate for the analysis of total Sb in the samples. It is important to remark that the measurement frequency of such FIA system was 28 h^{-1}.

Flores et al. [18] employed hydride generation-atomic absorption spectrometry for the speciation of Sb in commercial samples of injectable drugs used for leishmaniasis treatment. The procedure is based on the selective hydride generation from Sb(III) in presence of citric acid, used to inhibit the formation of hydride formation from Sb(V). Thus, after addition of a suitable reductant, total Sb is determined and Sb(V) is obtained by difference. Operating at optimum conditions the authors achieved a detection limit of 1.5 ng of Sb, which demanded high dilution factors for analysis since the amount of Sb in the samples varied from 83.5 to 110.3 mg ml^{-1}.

Rath et al. [16] employed the selective reaction between Sb(III) and bromopyrogallol red (BPR) to develop a method for antimony speciation in commercial drugs used in the treatment of leishmaniasis. In a such manual method, Sb(III) reacts with BPR decreasing the absorbance of the solution at 560 nm. At the same conditions, Sb(V) does not react with BPR. In order to determine Sb(V) in the sample, the authors utilized similar approach employed by Flores et al. [18], reducing all Sb(V) to Sb(III) and posterior calculation of Sb(V) by difference. The same research group developed a FIA system for Sb(V) determination with rhodamine B in the same samples. The set-up included a liquid–liquid extraction unit for the measurement of the complex [15].

Recently, a FIA/spectrophotometry system was used for Figueiredo et al. [17] to determine Sb(III) and total Sb in antileishmanial drugs. The method was based on the SbH_3 generation and its posterior reaction with $KMnO_4$ solution which has your color intensity diminished due to reduction by stibine.

In this sense, an interesting paper was published by Petit de Peña et al. [20] regarding to Sb speciation in biological tissues. In this work the authors developed a fully automated procedure for extraction and speciation analysis of Sb by HG AAS. Acetic and sulfuric acids were selected among other acids for extraction of Sb(III) and Sb(V), respectively, and L-cysteine was employed for Sb(V) reduction to Sb(III) in order to make possible stibine generation and Sb detection in the quartz cell. The developed methodology was applied in the Sb speciation in biological tissues like cattle liver and hamster blood and kidney. The detection limits achieved were 1 μg l^{-1} for Sb(III) and 0.5 μg l^{-1} for Sb(V).

The aim of this work was to develop a reversed FIA system for the speciation of Sb in drugs used in the treatment of leishmaniasis also exploring the reaction of the Sb(III) ions with BPR in an organized medium containing Triton X-100. A reversed FIA approach was utilized for reagent saving and due to the high absortivity of BPR solutions. In a normal FIA configuration the high absortivity of BPR would lead to a high blank absorbance, strongly affecting the detector operation and thus increasing the baseline noise. In a reversed FIA mode, such problem is not verified.

2. Experimental

2.1. Apparatus

The set-up consisted of a Femto 700 Plus (São Paulo, Brazil) UV–vis spectrophotometer equipped with a Hellma (Jamaica, NY, USA) standard glass flow-cell of 80-μl internal volume and 10-mm optical path. The instrument was set at 555 nm for all absorbance measurements. A Micronal B-332 (São Paulo, Brazil) peristaltic pump, furnished with flexible PVC tubes (Tygon®), was used to propel all solutions and a Rheodyne 5041 six-port valve was employed to inject the reagents mixture into the system. The manifold was built up with PTFE tubes with 0.8 mm bore and PEEK plastic connections.

All pH measurements were carried out in an Analyzer 300 pHmeter equipped with a combined glass electrode (Ag/AgCl as reference).

Flame atomic absorption measurements were carried out in a Perkin-Elmer (Norwalk, CT, USA). Analyst 100 spectrometer equipped with an antimony hollow cathode lamp. The instrument was operated at optimum conditions suggested by the manufacturer (lean blue air-acetylene flame, wavelength = 217.6 nm and slit width = 0.2 nm).

2.2. Reagents and solutions

All solutions were prepared with analytical grade reagents and high purity deionized water, obtained in a Simplicity Milli-Q (Millipore, Saint Quentin Yvelines, France) water purification system.

Antimony solutions. Antimony(III) solutions were prepared daily by adequate dilution of a 1000 mg l^{-1} Sb(III) stock standard solution that was obtained by dissolution of 2.8082 g of $K(SbO)C_4H_4O_6{\cdot}0.5H_2O$ with 50 ml of a 2 mol l^{-1} HCl solution and posterior dilution to 1000 ml in a volumetric flask. As the same way, Sb(V) standard solutions were prepared by dilution of a 1000 mg l^{-1} stock solution prepared by dissolution of a 2.1592 g of $KSb(OH)_6$ in around 250 ml of purified water under heating of 60 °C. After total dissolution of the solid, the solution was left to achieve ambient temperature and the volume was made up to 1000 ml in a volumetric flask.

BPR reagent solution. A 500 mg l^{-1} stock solution of BPR was prepared by dissolving 50 mg of BPR (Acros, USA) in exactly 100 ml of purified water. The solution was stored in a dark flask and maintained in the dark. Under these conditions the solution remains stable for 2 weeks, at least. All solutions of BPR used throughout the experimental work were obtained by convenient dilution of the stock solution.

Triton X-100 solution. A 5 g l^{-1} Triton X-100 solution was prepared by mixing 0.5 g of Triton X-100 (Vetec, Rio de Janeiro, Brazil) with around 80 ml of purified water. After obtaining a homogeneous solution the volume was filled up to 100 ml in a volumetric flask.

Carrier solution. A concentrated buffer solution of NaH_2PO_4/Na_2HPO_4 with pH 6.8 (total concentration 1 mol l^{-1}) was prepared by dissolving 15 g of NaH_2PO_4 (Vetec, Rio de Janeiro, Brazil) and 17.7 g of Na_2HPO_4 (Vetec, Rio de Janeiro,