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Peroxidase immobilized on Amberlite IRA-743 resin for on-line spectrophotometric detection of hydrogen peroxide in rainwater

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Abstract

The importance of atmospheric hydrogen peroxide (H_2O_2) in the oxidation of SO_2 and other compounds has been well established. A spectrophotometric method for the determination of hydrogen peroxide in rainwater is proposed. This method is based on selective oxidation of hydrogen peroxide using an on-line tubular reactor containing peroxidase immobilized on Amberlite IRA-743 resin. The hydrogen peroxide in the presence of phenol, 4-aminoantipyrine and peroxidase, produces a red compound ($\lambda = 505$ nm). Beer's law is obeyed in a concentration range of 1–100 μmol l^{-1} hydrogen peroxide with an excellent correlation coefficient ($r = 0.9991$), at pH 7.0, with a relative standard deviation (R.S.D.) <2%. The detection limit of the method is 0.7 μmol l^{-1} (4.8 ng of H_2O_2 in a 200 μl sample). Measurements of hydrogen peroxide in rain samples were carried out over the period from November 2003 to January 2005, in the central area of the Juiz de Fora city, Brazil. The concentration of H_2O_2 varied from values lower than the detection limit to 92.5 μmol l^{-1}. The effects of the presence of nonseasalt (NSS) SO_4^{2-}, NO_3^- and H^+ in the concentration of hydrogen peroxide in the rainwater had been evaluated. The average concentrations of H_2O_2, NO_3^-, NSS SO_4^{2-} and SO_4^{2-} are 23.4, 18.9, 7.9 and 10.3 μmol l^{-1}, respectively. The pH values for 82% of the collected samples are greater than 5.0. The spectrophotometeric method developed in this work that uses enzyme immobilized on the resin ion-exchange compared with the amperometric method did not present any significant difference in the results.

Keywords: Hydrogen peroxide; Rainwater; Peroxidase immobilized; Flow-injection system

1. Introduction

The accurate determination of hydrogen peroxide (H_2O_2), is becoming of great importance in atmospheric and biochemical processes. H_2O_2 is a significant species in photochemical smog as a chain terminator and as an indicative of hydroperoxyl radical ($HO_2^\bullet$) concentrations. Atmospheric hydrogen peroxide is formed from the interactions of hydroperoxyl and hydrated hydroperoxy ($HO_2^\bullet HO_2$), which are produced by the photochemical reactions of atmospheric trace gases, such as ozone and volatile organic compounds [1]. Significant emissions of H_2O_2 from direct, natural or anthropogenic sources are not known [2]. Photochemical models suggest that H_2O_2 should be present in both polluted and clean air. Increase in H_2O_2 may result in a significant increase in the rate and extent of acidification of the rain [3]. The potential of H_2O_2 as a major oxidant leading to H_2SO_4 generation in the aqueous phase of the troposphere occurs through the reaction between dissolved sulfur dioxide (SO_2) and H_2O_2 [1–6]. This reaction is relatively rapid even at pH values below 5.0, whereas the oxidation of SO_2 by other oxidants, such as O_3 and O_2 in presence of Fe and Mn as catalysts is retarded in acidic atmospheric waters [7,8]. During the last two decades, a number of investigations have been carried out concerning the measurement of H_2O_2 in the atmospheric gas and liquid phases [1–4,9–14].

The availability of H_2O_2 in both the gas phase and the aqueous phase has become the focus of greater attention in the last decades [15]. Different research groups have also studied the effects of several meteorological and chemical factors on the concentration of H_2O_2 in cloud and rainwater [2,3,16].

Actually, chemical sensors with the ability of continuously sensing analytes attract considerable attention and many appli-

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cations have appeared in the literature [17–19]. Many analytical methods are described in literature to quantify the presence of H_2O_2 in flowing solution, including spectrophotometry [1,20], fluorometry [2,21], amperometry [4,22], voltammetry [23] and chemiluminescence [10,24].

Some enzymatic techniques for the determination of hydrogen peroxide are actually available and include the use of peroxidase or catalase [4,25–28]. These enzymes are highly specific for the oxidation of hydrogen peroxide. Other possibility is the direct electrochemical detection of H_2O_2 using electrodes or microelectrodes such as gold, platinum or glassy carbon modified [4].

Strategies have been undertaken to adapt quantification methods to the range of sample concentrations normally taking in account the precision, speed and with low cost. For the analytical methods involving enzymes the reduction in the determination costs is generally associated with reduced optimized enzyme consumption. This has been achieved in our research group by immobilization of the catalase in the analytical system for determination of the hydrogen peroxide in rainwater and glucose oxidase for determination of glucose in blood samples [4,17].

Recently, several ion-exchange resins have gained considerable significance not only for separation purposes but also as carriers of catalytic active substances. Until now, quite an attention has been paid to their application for immobilization of enzymes [17,25–28]. The resins ought to meet many requirements. Their porous structure must be strong enough to withstand an enhanced pressure usually applied in forced flow bioreactors. Besides, the membrane material must be chemically and physically resistant. These requirements can be met in many aromatic and aliphatic polyamides. Therefore, resin prepared from these polymers would be suitable carriers for immobilization of enzymes [29]. The covalent binding of the enzyme with the polymer matrix is one of the most promising methods for immobilization.

In present work, we describe a versatile method for spectrophotometric determination of micromolar hydrogen peroxide in rainwater samples, using an on-line tubular reactor containing peroxidase immobilized on resin (Amberlite IRA-743). In the next sections the authors present a new method of enzyme immobilization for applications to real samples. The authors have also studied the influence of SO_2 and NO_x on the H_2O_2 levels. Practical application of the method involved the quantification of hydrogen peroxide present in rainwater collected in Juiz de Fora city, Brazil.

2. Experimental

2.1. Preparation of the enzymatic reactor

The procedure adopted to immobilize the peroxidase enzyme is fast and very simple [17]. Amberlite IRA-173 resin (originally manufactured for applications involving boron extraction) was selected as support, which has active amine groups in its chemical structure. The immobilization is presented schematically in Fig. 1. The enzyme immobilization process begins with

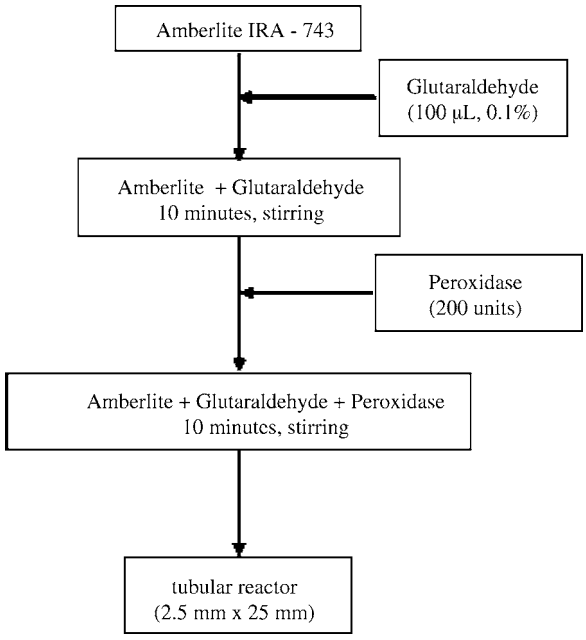

Fig. 1. Schematic diagram showing the immobilization of the peroxidase on Amberlite IRA-743.

the addition of 100 μl of glutaraldehyde 0.1% on 250 mg of resin and this mixture is stirred for 10 min. After, 200 units of enzymes are introduced into the mixture and stirred for more 10 min. In the next step, the resin is transferred to a tygon tube (2.5 mm of i.d. and 25 mm long length) having one of its extremities closed with a thin layer of glass wool for the assembling of the reactor. Finished this step, the other extremity of the tube was also closed with glass wool. To adapt the enzymatic reactor to the flow FIA system the tubes (with 0.5 mm of i.d.) were fixed under pressure in each one of its extremities with of a small pieces of silicone tubes (1.3 mm i.d. and 5 mm long length). In the last step, the reactor was washed with 10 mmol l^{-1} phosphate buffer solution (pH 7.0) to remove the excess of peroxidase.

2.2. Reagents and chemicals

All reagents used were of analytical grade and were used as received. Hydrogen peroxide, mono- and di-hydrogen phosphate were obtained from Merck (Darmstadt, Germany). Phenol with greater than 99.5% purity and 4-aminoantipyrine were obtained from Aldrich (Milwaukee, USA). Solutions were prepared with water previously distilled and purified in a nanopure system. Commercial peroxidase (EC 1.11.1.7-115 U mg^{-1}) was obtained from Sigma (St. Louis, MO). The Amberlite IRA-743 ion-exchange resin and glutaraldehyde were obtained from Aldrich (Milwaukee, USA). Aqueous stock solutions of phenol and 4-aminoantipyrine were prepared using 0.10 mol l^{-1} pH 7.0 mono- and di-hydrogen phosphate. They were stored at 4 °C when not utilized. Diluted solutions of hydrogen peroxide were prepared daily using deionized water.

2.3. Instrumentation

In this work, a flow system was employed. The solutions were propelled by pressurization, utilizing an aquarium air pump to avoid the undesirable pulsation observed when peristaltic pumps are employed [4,17,30]. Control of the flow rate was done by adaptation of the aquarium valve commonly used to pinch a tygon tube inserted in the line. Teflon tubing of 0.5 mm i.d. was used throughout the flow system. The flow system used during the development of this work consisted of two lines, in first the reagents were added in the system, in the second the sample was inserted and mixed in the reaction coil with the reagents for color generation. A Shimadzu U.V.1601 PC spectrophotometer operated from a microcomputer was used for colorimetric assays. Glass cuvettes with 1 cm optical path length and 1.5 ml volume were obtained from Hellma Ltda (Concord, ON). To control the temperature was utilized a thermostatic bath (THERMOMIX 18 BU B. Braun Biotech. International). To be possible control the temperature of all assays with a precision of 0.1 °C, these experiments were done in a thermal stabilized room. The system is constituted of an aquarium air pump, a pinch valve, sampling loop, a tubular reactor (Ø = 0.25 cm and 2.5 cm of length) with chemically immobilized peroxidase on Amberlite IRA-743 resin, a cuvettes and the spectrophotometer.

The measurements of pH in rainwater samples were performed as soon as possible after sampling with a potentiometer (Digimed DM 20) using a pH meter with a glass electrode combined with a reference electrode (Ag/AgCl), calibrated with Merck standard buffer solutions (pH 4 and 7). A radiometer Digimed DM 31 conductivity meter and electrode was used for conductivity measurements.

The concentrations of the major ions Na^+, NO_3^- and SO_4^{2-} were measured in the studied rainwater samples using a capillary electrophoresis system with an automatic sampler and a spectrophotometric detector (CE Hewlett-Packard) with indirect detection in 214 nm (Na^+) and 375 nm (NO_3^- and SO_4^{2-}) [31].

Differentiation of sea-salt and nonseasalt components is essential for many studies of precipitation chemistry. The seasalt fraction of a particular chemical constituent, C_{SS}, of a precipitation sample was calculated from:

$$C_{SS} = \left(\frac{C_{SW}}{Ref_{SW}}\right) \times Ref_{sample} \quad (1)$$

where C_{SW} is the concentration of C in seawater, Ref_{SW} is the concentration of the reference species (Na^+) in seawater and Ref_{sample} is the concentration of the reference species in the precipitation sample. The nonseasalt fraction C_{NSS} is calculated from:

$$C_{NSS} = C_T - C_{SS} \quad (2)$$

where C_T is the total concentration of C in the precipitation sample [32]. Nonseasalt (NSS) sulfate concentrations were estimated using seawater sodium/sulfate, assuming all sodium present in these rainwater samples was from sea-salt, because the analyzed samples had $[Cl^-]/[Na^+]$ ratio of 0.86 [33].

2.4. Procedure

Colorimetric assay was used to measure the concentration of hydrogen peroxide using peroxidase chemically immobilized in Amberlite IRA-743 resin. With phenol and 4-aminoantipyrine concentrations present in sufficient quantity, the rate of color generation (antipyrilquinoneimine dye) at 505 nm is proportional to the rate of hydrogen peroxide consumption. The generation of color by reaction of phenol (PhOH) + 4-aminoantipyrine (Am-NH_2) + H_2O_2 in the presence of the peroxidase (PEO) may be represented by the following total reaction [34]:

$$PhOH + Am\text{-}NH_2 + 2H_2O_2 \xrightarrow{PEO} Dye + 4H_2O$$

2.5. Sample collection

Rainwater sample collection was carried out over the period from November 2003 to January 2005, in the central area in the Juiz de Fora city, Brazil. The sample collection site is located on the Rio Branco Avenue. The rainwater samples were collected and analyzed rapidly after collection or preserved by freezing and stored at −20 °C. After collection, the samples were filtered through a 0.45 μm cellulose acetate membrane (MF filter, from Millipore Co., Bedford, MA) and analyzed immediately. Meteorological data (solar intensity, relative humidity and precipitation volume) were obtained from the local meteorology station.

3. Results and discussion

3.1. Immobilized peroxidase

The specific activities of free and immobilized PEO were determined spectrophotometrically (at 505 nm) on static condition. The method is based on the change of the solution color resulting from the oxidation of 4-aminoantipyrine, phenol and hydrogen peroxide, in presence of the enzyme peroxidase. The properties of PEO immobilized onto Amberlite IRA-743 modified with 0.1 wt.% glutaraldehyde were studied to determine the best pH, temperature optimum and efficiency for the complete oxidation of the hydrogen peroxide, a fundamental condition for applications in the determination of hydrogen peroxide in rainwater. The activity of hydrogen peroxide at different pH values conditions, varying from 4.0 to 9.0, for injection of 200 μl of 1.5×10^{-5} mol l^{-1} hydrogen peroxide. The optimum pH of PEO immobilized in the tubular reactor was 7.0. The activity of peroxidase at different temperatures for injection of 200 μl of 1.5×10^{-5} mol l^{-1} hydrogen peroxide is presented in Fig. 2a. In this experiment, the temperature of the system (enzymatic reactor and bobbin of reaction coil) was controlled using a thermostatic bath. The optimum temperature for PEO immobilized onto Amberlite IRA-743 was 40 °C.

To examine the efficiency of the tubular reactor containing immobilized peroxidase on the resin, experiments involving consecutive injections of hydrogen peroxide solutions were performed. Response of spectrophotometric sensor for injection of

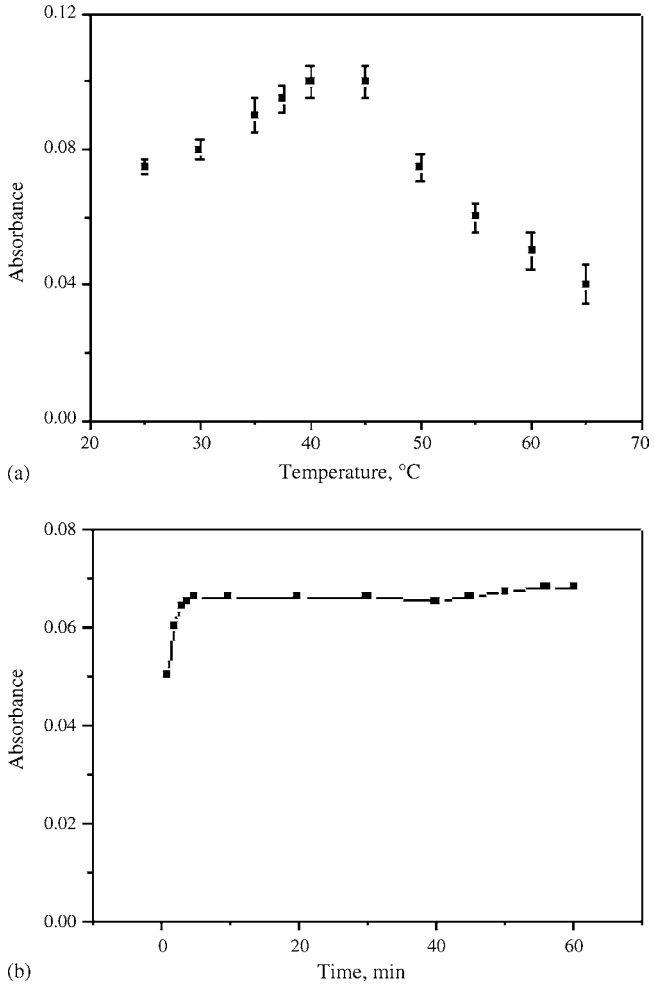

Fig. 2. Activity of the peroxidase (a) at different temperature values between 20 and 60 °C for injection of 1.5×10^{-5} mol l^{-1} hydrogen peroxide and (b) stabilities of the immobilized peroxidase in Amberlite IRA-743 resin for repetitive injections of 1×10^{-5} mol l^{-1} hydrogen peroxide. Conditions—0.10 mol l^{-1} phosphate buffer (pH 7.0); sample volume: 200 μl; flow rate: 1.5 ml min^{-1}; λ: 505 nm.

200 μl 1.0×10^{-5} mol l^{-1} hydrogen peroxide with immobilized PEO was obtained. Fig. 2b shows the stability of immobilized PEO in Amberlite IRA-743 resin. An important characteristic observed of the immobilized enzyme is the storage stability in with time. Reactors prepared have presented high stability for at last 5 days under intense use in flowing solutions, when submitted the 100 injections of 200 μl 1.0×10^{-5} mol l^{-1} hydrogen peroxide. After this period, a decrease on the order of 30–45% on the enzyme activity has been observed. When applied in rainwater the enzymatic reactor it has shown a loss of enzymatic activity after 50 injections, requiring the construction of a new reactor. When not in use, the reactors were stored in a freezer at −20 °C.

3.2. Optimization of the FIA system

The influence of parameters, such as flow rate and sample volume was studied. The spectrophotometric response for injections of 200 μl 1.0×10^{-5} mol l^{-1} hydrogen peroxide as a function of the flow rate, varied from 0.5 to 5.0 ml min^{-1} was evaluated. For high flow rates the residence time of the hydrogen peroxide in the reactor is shorter, which causes a significant decrease on the analytical sensitivity. A flow rate of 1.5 ml min^{-1} was chosen as the most favorable, since it combines good reproducibility, high throughput, and lower consumption of carrier solution.

The influence of the sample volume on the analytical signal was also evaluated. Loops with internal volumes varying from 50 to 250 μl were tested. When the volume of the sample is increased, the spectrophotometric signal increases, but the time required for each analysis also increases. A volume of 200 μl was chosen as the working volume in subsequent experiments. For all the volumes studied the peroxidase immobilized in the tubular reactor was sufficient to promote the selective oxidation of hydrogen peroxide.

3.3. Chemical variables

Those reagents which directly influence formation of the absorbing species were evaluated to determine optimum concentrations that provide high absorbance without utilization of a large excess of reagents. Various concentrations of phenol, peroxidase and 4-aminoantipyrine were evaluated. Fig. 3a–c shows the spectrophotometric signal for injection of 200 μl 1.0×10^{-5} mol l^{-1} hydrogen peroxide increases when phenol, peroxidase and 4-aminoantipyrine concentrations are increased. Dramatic changes are observed initially followed by more gradual increases. Peroxidase and 4-aminoantipyrine gave no increase above 150 U ml^{-1} and 0.5 mmol l^{-1}, respectively. For phenol from 1.5 to 4.0 mmol l^{-1} an increase of 14% was verified. The optimum phenol, 4-aminoantipyrine and peroxidase concentrations found were 1.87 mmol l^{-1}, 0.5 mmol l^{-1} and 120 U ml^{-1}, respectively. A mixed reagent which includes phenol, 4-aminoantipyrine, peroxidase, and buffer when prepared daily yields results comparable to those obtained with the multiple reagent line FIA system.

Organic hydroperoxides such as methyl hydroperoxide (CH_3O_2H) and peroxyacetic acid [$CH_3C(O)O_2H$] are present in samples obtained from the aqueous phase of the atmosphere, although in lesser extent in comparison with hydrogen peroxide. In the present work, no spectrophotometric response for hydroperoxide acid was observed, and for peroxyacetic acid small absorbance peaks was gotten. These results attest the high selectivity of the phenol/4-AAP colorimetric system for H_2O_2 determination and its potentiality for application in environmental samples.

3.4. Calibration plot

The calibration plot showed the proportionality between the absorbance and hydrogen peroxide concentrations for successive injections of 200 μl hydrogen peroxide from 1 to 100 μmol l^{-1}. The hydrogen peroxide concentration can be cal-

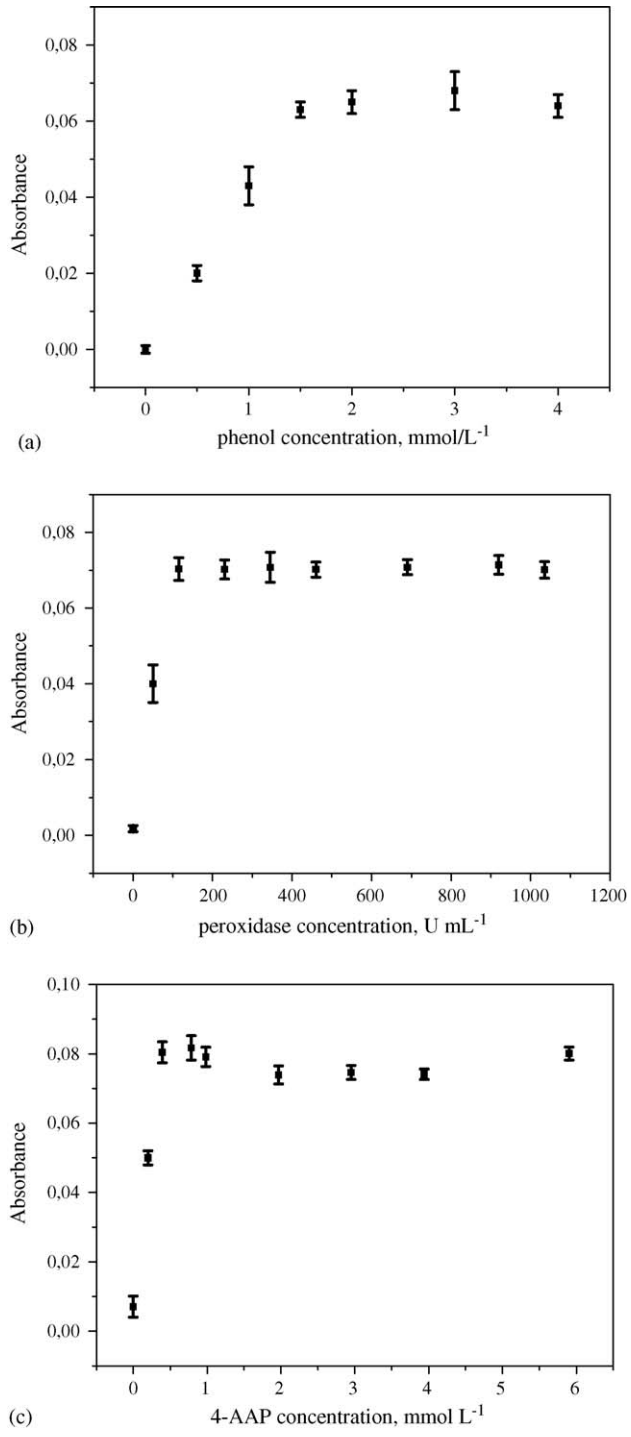

Fig. 3. Effect of the concentrations of (a) phenol; (b) peroxidase; and (c) 4-aminoantipyrine involving injections of 1×10^{-5} mol l^{-1} hydrogen peroxide, standard deviation of data are shown as error bars. Conditions—0.10 mol l^{-1} phosphate buffer (pH 7.0); sample volume: 200 μl; flow rate: 1.5 ml min^{-1}; λ: 505 nm.

culated using

$$\text{Abs} = 7665.1\,[\text{hydrogen peroxide}]\,(\text{mol}\,l^{-1}) - 0.00333 \qquad (3)$$

The correlation coefficient for linear regression was 0.9991. The detection limit for the conditions adopted in present study was found as 0.7 μmol l^{-1} (three times the standard deviation of the blank) [35].

3.5. Determination of H_2O_2 in rainwater

To quantify hydrogen peroxide in rainwater, the samples to be analyzed were mixed on-line with buffer solution, used as the carrier solution. This assay uses phenol, 4-aminoantipyrine, and peroxidase chemically immobilized in Amberlite IRA-743 resin as color-generating substrates. The spectrophotometeric method developed in this work that uses enzyme immobilized in the resin ion-exchange compared with the amperometric method did not present any significant difference in the results. The H_2O_2 concentration values determined by the two FIA methods in standards and in five samples, were compared using the linear regression procedure and the paired *t*-test [35]. The confidence interval for the slope and intercept are (0.98 ± 0.05) and (0.02 ± 0.03) μmol $^{-1}$ l^{-1}, respectively, for a 95% confidence level. Taking into account these results, no significant differences between the methods were observed, which strongly indicates the absence of systematic errors. The rainwater data obtained is mainly discussed, once a great number of samples colleted (60) in this monitoring site enabled us to perform a statistical analysis of the analytical data. A summary of the total data obtained in the sampling site for period of November 2003–January 2005 is given in Table 1. The average concentrations of H_2O_2, NO_3^- and SO_4^{2-} are 26.5, 18.9 and 10.3 μmol l^{-1}, respectively. These reported data indicate that the hydrogen peroxide levels found in the studied area were compared with those obtained by other investigators in different sites [1–4,11]. The areas with higher concentrations of H_2O_2 are regions with a high photochemical activity due to intense levels of solar radiation, urban and highly industrialized regions or rural areas with a great-scale transport of polluted urban air. Valley to stand out that the significant emissions of hydrogen peroxide from natural or anthropogenic sources do not exist, therefore, all the hydrogen peroxide will be considered as formed by photochemical reactions in the atmosphere (predominantly forming coupling of two hydroperoxyl radicals) [2]. Since the level of solar radiation in the area investigated in present study is relatively high, the concentrations of H_2O_2 in the liquid phase will be high. On the other hand, the presence of SO_2 and NO_x tends to inhibit the formation of H_2O_2 by scavenging free radical species from the air [3]. The reaction of these radicals $HO_2^\bullet$ with nitric oxide produces a significant decrease in the generation of H_2O_2.

Fig. 4 shows that hydrogen peroxide levels were highest during the spring (September–November) and summer (December–February) months and lowest in the autumn and winter periods. There is a seasonal tendency for hydrogen peroxide levels, which indicates a certain relationship with the annual variation of solar radiation. This can be explained by the photochemical origin of $HO_2^\bullet$ in the gas phase and the dominant aqueous-phase pathway for the generation of H_2O_2 by the self-reaction of $HO_2^\bullet$, derived mainly from gas phase scavenging. The higher solar intensity during spring and summer months is favorable to the generation of H_2O_2.

Table 1
Measurements of concentrations of chemical species in rainwater colleted in Juiz de Fora, Minas Gerais, Brazil between November 2003 and January 2005

	H_2O_2 ($\mu mol\,l^{-1}$)	pH	T (°C)	SO_4^{2-} total ($\mu mol\,l^{-1}$)	NSS SO_4^{2-} ($\mu mol\,l^{-1}$)	NO_3^- ($\mu mol\,l^{-1}$)
Minimum	1.1	4.4	14.5	0.7	0.1	1.8
Maximum	92.5	7.1	26.8	32.5	26.6	61.7
Mean	23.4	5.2	20.8	10.3	7.9	18.9
N	60	60	60	60	60	60

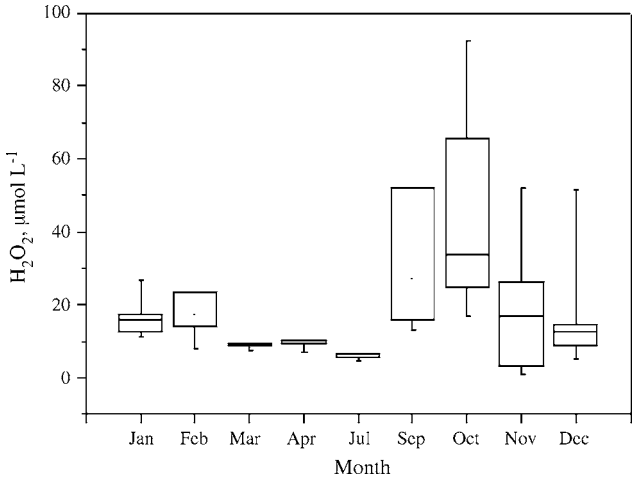

Fig. 4. Box–Whisker plots of the monthly variations for H_2O_2 concentration.

Due to the high solubility of gaseous H_2O_2 in water [2], washout and rainout processes have been considered as the dominant removal pathway for atmospheric H_2O_2; thus, the amount of rainfall can affect the levels of hydrogen peroxide in the gas and aqueous phases. Fig. 5 shows the H_2O_2 concentration in rainwater measured versus the amount of rainfall, where can be observed a certain tendency to have higher levels of H_2O_2 associated with a small quantity of rainfall. This same tendency was pointed out by different authors [2,3,14], who have also observed higher precipitation rates associated with lower H_2O_2 concentration in rain; this fact confirms the important role played by rainwater dilution.

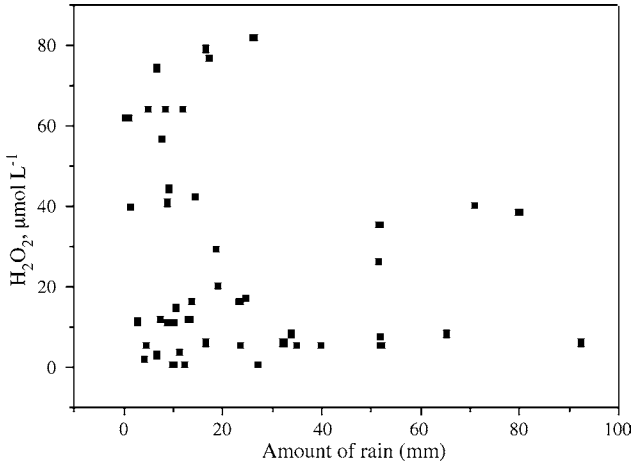

Fig. 5. Dependence of the H_2O_2 concentration on the amount of rainfall.

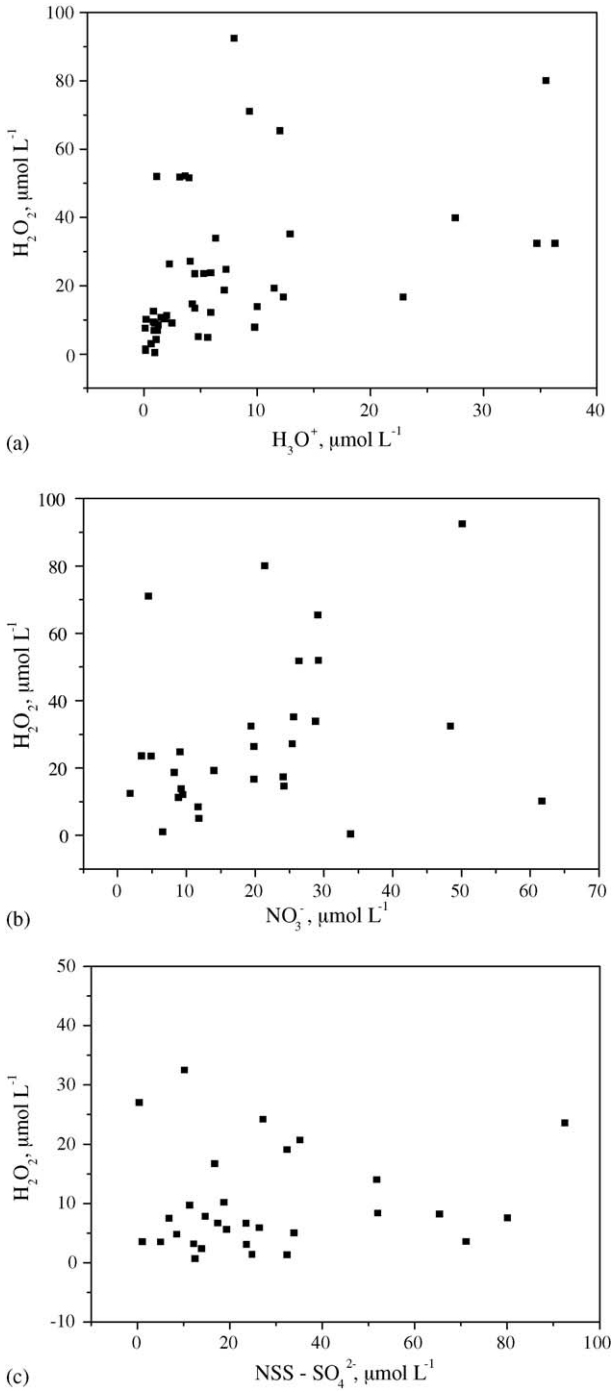

Fig. 6. Effects of major chemical species on the concentrations of H_2O_2 in rainwater: (a) hydrogen ion; (b) nitrate; and (c) nonseasalt (NSS) sulfate.

In order to study the effect of major chemical species on the H_2O_2 rainwater concentration, Fig. 6a–c shows the correlation between hydrogen peroxide and chemical species determined. In the present study, it has been observed that the higher concentrations of hydrogen peroxide corresponded with nitrate concentrations lower than 30 $\mu mol\,l^{-1}$, hydrogen ion levels lower than 30 $\mu mol\,l^{-1}$ and sulfate concentrations inferior to 40 $\mu mol\,l^{-1}$. All these samples, with medium levels of H_2O_2 in the range 1.1–92.5 $\mu mol\,l^{-1}$ and high levels of SO_4^{2-}, NO_3^- and H^+ ion, indicated isolated pollution events.

The H_2O_2 is the principal oxidizing agent for SO_2 in the cloud and rain droplets at pH <5, whereas O_3 the dominant oxidant at pH >5; thus, the determination of the pH values in the studied rainwater samples was demonstrated to be useful in elucidating fundamental for the role of ozone and hydrogen peroxide in the conversion of sulfur dioxide in sulfate [7,8]. The pH values for 82% of the collected samples were greater than 5.0; therefore, in these cases, the oxidation of SO_2 by O_3 is dominant, as can be seen in Fig. 6a. In the rest of the collected samples (18%), the pH was lower than 5; thus, the oxidation by H_2O_2 was the dominant pathway in the other cases. If the nonseasalt sulfate found in rainwater were solely derived from the interaction of H_2O_2 with SO_2, a negative correlation between H_2O_2 and sulfate in rainwater would exist. However in this work, a weak inverse correlation was observed between H_2O_2 and NSS SO_4^{2-} (Fig. 6c).

The NO_x can mediate in both formation and decomposition of H_2O_2 in the atmosphere [29]. Nitrate ion, as the final product of NO_x oxidation can be negatively related with H_2O_2 in rainwater. Fig. 6b shows that any direct correlation can be obtained from this study. The lack of a good inverse correlation between nitrate and hydrogen peroxide supports the conclusion that H_2O_2 was not the only oxidant for NO_x in the studied area.

4. Conclusions

This work demonstrated the potentiality of the spectrophotometric method using peroxidase immobilized in a tubular reactor associated with FIA techniques, for detection of hydrogen peroxide in rainwater samples. The very high sensitivity provided by technique, combined with the high activity of the immobilized peroxidase in Amberlite IRA-743, allows us to work with small sample volumes and at low concentrations. It has yielded similar results to the amperometric method. Good reproducible and constant results were obtained both for synthetic solutions of hydrogen peroxide as well as for blood samples, indicating the absence of the poisoning process of the tubular reactor [4,17]. This work is the first contribution for the acknowledgement of the hydrogen peroxide levels in the Juiz de Fora city, Brazil. The concentrations of H_2O_2 and other major chemical species in rainwater samples were measured between November 2003 and January 2005. The hydrogen peroxide levels were highest during the spring and summer months, and the maximum concentrations were recorded during October 2004. No strong correlation between the concentration of H_2O_2 and the pollutant species (SO_4^{2-}, NO_3^- and H^+) was evident.

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