Automated Monosegmented System for Hydrogen Peroxide Determination at Low Levels

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Abstract

Hydrogen peroxide (H_2O_2) is a chemical agent widely used as packaging sterilant material. An automated monosegmented flow system for the determination of residual H_2O_2 in rinse water of plastic bottles is proposed. The system is based on the kinetic reaction of Tiron with H_2O_2, which is catalyzed by cobalt and used for H_2O_2 determination. High sensitivity was achieved at pH = 13 using Tiron and cobalt at concentrations of 5 x 10^{-4} mol l^{-1} and 10 μg l^{-1}, respectively. The calibration graph was linear in the range 0 - 6.0 mg l^{-1} (r = 0.9902). The detection and quantification limits were 19 and 63 μg l^{-1}, respectively. RSD was 5% for repeatability and 9% for reproducibility (n = 9 for both). With this system it was possible to perform 100 determinations/hour, using only 0.53 μg of Tiron per determination.

Keywords Hydrogen peroxide, monosegmented system, tiron, flow analyses, spectrophotometric determination.

1. Introduction

Hydrogen peroxide has been recognized as a bactericidal agent for many years and it is used in several applications to decontaminate equipment and materials for aseptic packaging[1-3]. In production of aseptically packaged foods, decontamination of the packaging material is of utmost importance to ensure the microbiological safety and stability of the product for the purpose of controlling the growth of microorganisms [4].

The Food and Drug Administration (FDA) approved the use of H_2O_2 as a sterilant since 1981[5]. However, the residual H_2O_2 present in the filled product must be controlled, since it can cause cancer. This has been confirmed in mice whose duodena were affected after H_2O_2 administration in drinking water at concentrations between 0.1% and 0.4%[6].

Due to the importance of H_2O_2 determination, many spectrophotometric methods have been described in the literature but most of them employ long and complex procedures as well as unstable reagents. A list of some applications and its analytical characteristics is presented in Tab. 1. It is interestingly pointed out that no application presented in this Table is related to automated system.

In order to improve the performance of the method related to sample handling, time and reagent consumption, selectivity and/or sensitivity, the use of flow analysis[13] seems to be excellent.

In this way, Pasquini and Oliveira[14] proposed an elegant automated system for ammonia, phosphorus and chromium (VI) determinations in which the sample (natural water) was inserted between two air bubbles, decreasing radically the sample dispersion into the system with consequent increase in sensitivity. The sensitivity can be still improved in these systems when catalytic reactions are employed[15].

In addition, unstable reactions/reagents can be used when automated systems are employed because these systems permit that measurements are performed at non steady-state conditions. An example of unstable reactions is the oxidation of Tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid, disodium salt monohydrate) with H_2O_2 catalyzed by cobalt. This reaction was first applied by Bognár[16] only to synthetic samples and exploited by Arruda *et al.*[17] at higher pH values where unstable reaction conditions were attained, but with increase in sensitivity.

In this way, this work presents a development of an automated method for residual H_2O_2 determination (at μg l^{-1} level) in water from plastic bottles immediately after sterilization process, using a monosegmented system and exploiting a new application for the Tiron reaction.

2. Material and methods

In the spectrophotometric determination of H_2O_2 using the monosegmented system (Fig. 1), the transmission lines and loops were made of polyethylene (0.75 mm i.d.). A model 432 spectrophotometer (Femto, Brazil) set at 426 nm was used.

The monosegmented flow system employed a peristaltic pump (Ismatec IPC, Switzerland) and a homemade three-piece injector-commutator device made of polymethacrylate[18].The acquisition of data was performed by a personal microcomputer with a Pentium processor, employing a PCL 711-S (Advantech, Taiwan), and using a program written in Visual Basic 3.0. A Perkin-Elmer (USA) Model AAnalyst 300 atomic absorption spectrometer equipped with a deuterium background corrector was used in the lixiviation experiments.

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Table 1 Literatures related to the hydrogen peroxide determination.

Sample	Remarks	Ref.
Food	Extraction with 0.5% $KBrO_3$.	6
Water	Formation of complex between H_2O_2 and potassium titanium (IV) oxalate [$K_2TiO(C_2O_4)_2.2H_2O$].	7
Foodstuffs	Reduction of H_2O_2 by N, N-dimethyl-p-phenylenediaminedihydrochloride (DMPD), in presence of peroxidase.	8
Air	Formation of complex between H_2O_2 and pyridine-2,6-dicarboxylic acid and vanadate (V) in acid medium.	9
Water	Reduction reaction of copper (II) ion in presence of excess of 2,9-dimethyl-1,10-phenanthroline (DMP).	10
Food	Formation of complex between H_2O_2 and *o*-sulfophenylfluorone-titanium (IV).	11
Water	Formation of complex between H_2O_2 and leuco patent blue violet and peroxidase.	12

2.3. Samples – Bottles and Chemical Sterilants

Polyethylene terephtalate (PET) bottles for cold filling with 500 ml capacity were used in this experiment.

Three commercial sterilant products (SP) with H_2O_2 concentration of 35% (SP1), 6.9% (SP2) and 23% v/v (SP3) were used in this work. The sterilant SP2 additionally contains 4.7% v/v of peracetic acid and SP3 has 15% v/v of peracetic acid and 16% v/v of acetic acid. The first product (SP1) was used without dilution. The second and third products were used as a solution in which H_2O_2 concentrations were 0.021 and 0.46 v/v, respectively.

3. Procedure

3.1. Bottle Sterilization

The bottles were divided into three groups and each group contained 6 bottles. In the first group SP1 (filled until the top of a bottle) was used and the bottle rinsed for 10 seconds. In the second and third groups the same procedure was carried out but SP2 and SP3 solutions were used (see concentrations in Samples section).

For each of the above groups, 3 bottles were first rinsed with the sterilant, and then filled with distilled water. Hydrogen peroxide was then determined in the water. The other 3 bottles were also rinsed, and filled with distilled water as above, however in this instance a portion of water was discarded. Then the bottles were filled once again and the residual H_2O_2 was determined in this second water portion.

3.2. The Monosegmented System

For H_2O_2 determination the monosegmented system depicted in Fig. 1 was used. In the initial position (Fig. 1a) the sample was aspirated into loops L_{S1-2} (at 5 ml min^{-1}) while air fills loops L_{A1-2} (at 1.5 ml min^{-1}). The R_1, R_2 and R_3 reagents were pumped, mixed at B_1 coil (40 cm) and filled loop L_R (180 µL) while the carrier stream (C_s) was pumped to the spectrophotometric cell (D). When the injector-commutator (I) was switched to the alternative position (Fig. 1b), the volume of L_R loop was inserted into the volumes of L_{S1-2} loops (45+45 µL) and, then, both were inserted into the air stream from L_{A1-2} loops (45+45 µL). In this way, the carrier stream transported the sample zone protected by two air bubbles. Mixing between the sample and the reagent the color reaction development occurred in the B_2 reaction coil (100 cm). After 100 s from sample injection, the analytical signals were monitored spectrophotometrically at 426 nm and visualized from a display of computer. Different peak shapes were obtained in the monosegmented systems (Fig. 2) where firstly a high signal appeared. This signal was related to the first air bubble (see detail of Fig. 2) passing through the spectrophotometric cell. After this first peak, a signal (plateau) of the colored zone concerning the catalytic reaction was obtained, being this signal proportional to the hydrogen peroxide concentration in the sample. To end of a cycle, once again, a high signal was observed and related to the second air bubble.

3.3. Accuracy Check

The accuracy of the proposed method was assessed by using an alternative method described in the literature by Afsar *et al*[19]. The alternative method employed the indirect determination of H_2O_2, in which Fe (II) is oxidized to Fe (III) and the excess of Fe (II) is complexed with phenanthroline. In the procedure adopted, 100 µL of 0.01 mol l^{-1} $FeSO_4$ was added to 9 ml of sample, standards or blank and mixed for ten minutes. After addition of 500 µL of 0.01 mol l^{-1} phenanthroline to this mixture, 60 minutes were necessary for the reaction development. After this time the measurements were performed at 508 nm.

4. Results and discussion

4.1. System and Reagents Optimization

For reagent concentration optimization, some experiments described below were performed. The length of the sampling loop was adopted from a previous work described by Pereira-Filho and Arruda[20]. After previous experiment, the flow rates for the carrier stream, Tiron, NaOH, air and sample were 1.5, 1.0, 1.0, 1.5 and 5.0 ml min^{-1}, respectively, in order to increase the analytical frequency. On the other hand, the concentrations of Tiron and cobalt were optimized by employing classical multilinear regression[21].

In this way, a factorial design with two levels and two variables plus central point was performed (7 experiments). The concentrations of the variables were adapted from Pereira-Filho and Arruda[20]: 9×10^{-4} (-1 level), 1.8×10^{-3} (central point) and 2.7×10^{-3} mol l^{-1} (+1 level) for Tiron, and 10 (-1 level), 20 (central point) and 30 µg l^{-1} (+1 level) for cobalt. A polynomial of first order was proposed to relate the response Y (absorbance) to the normalized (-1 to +1) Tiron and cobalt concentration variables: $Y=b_0 + b_1T + b_2c$, where b_i = estimates of model parameters, T = Tiron concentration, c = cobalt concentration. After the calculation process, it was observed that cobalt concentration variation was not significant for the mathematical model and Tiron concentration presented a negative effect, due to the best results obtained with lower Tiron concentration. Therefore, the cobalt and Tiron concentrations adopted were 10 µg l^{-1} and 5×10^{-4} mol l^{-1}, respectively.

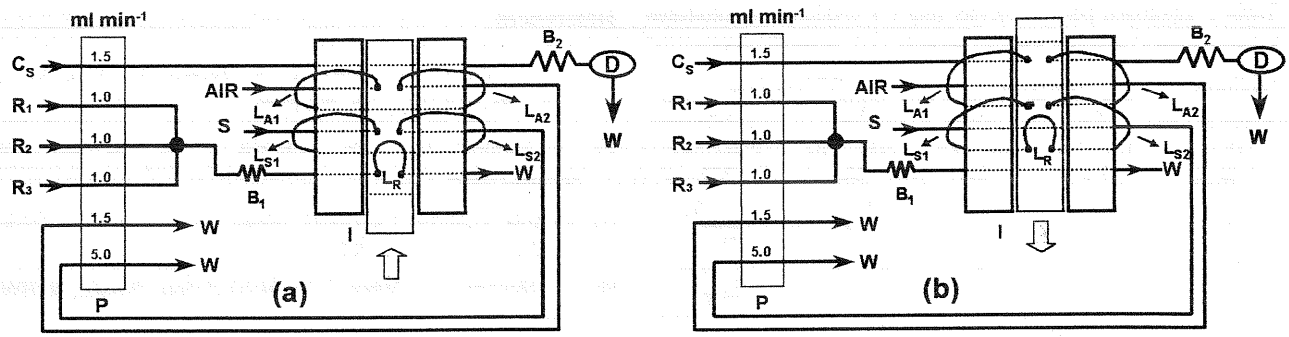

Fig. 1 Flow diagram for determination of H_2O_2. (a) Injector-commutator (I) in sampling position - R_1, R_2 and R_3: Tiron, cobalt and NaOH; C_S: carrier stream (0.014 mol l^{-1} HNO_3); D: detector (426 nm); L_{A1-2}, L_{S1-2} and L_R: air (45 μL each), sample (45 μL each) and reagent (180 μL) loops, respectively; B_1 and B_2: 40 and 100 cm coils; S: sample; AIR: air ; W: waste; P: peristaltic pump. (b) Injector-commutator in the injection position - The arrow indicates the next position of this piece. Other information, see text.

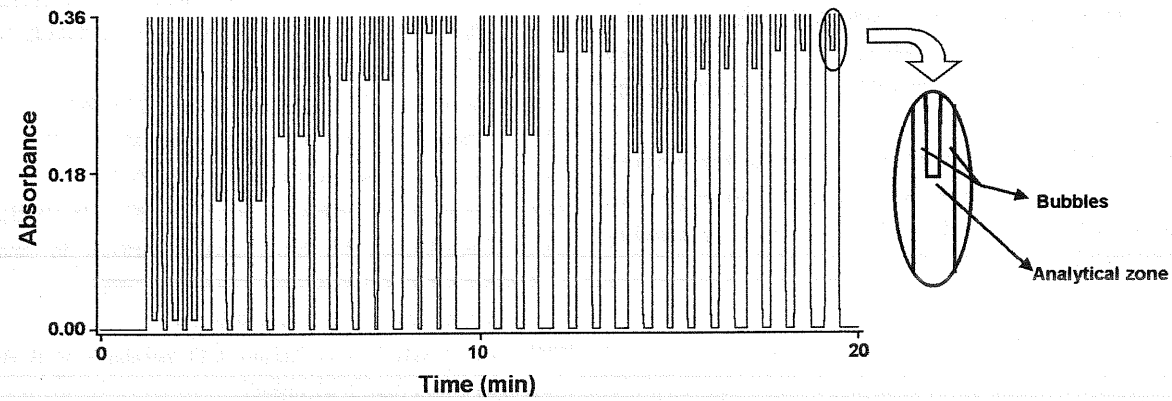

Fig. 2 Peak profiles obtained in the H_2O_2 determination. From left to right, blank, 1.0, 1.5, 2.0 and 2.5 mg l^{-1} in triplicate, followed by five samples also in triplicate.

4.2. Study of Lixiviation

In order to investigate the lixiviation of some metals from the wall of PET bottles by H_2O_2, some metal concentrations for copper, nickel, manganese and iron, in the rinse water from bottles with and without rinsing process were measured by FAAS. These analytes were determined because they can affect the selectivity of the spectrophotometric method when presented at high concentration. However, in the rinse water used, the concentrations of these metals were below the detection limits of FAAS (0.06, 0.05, 0.08 and 0.10 mg l^{-1} for Cu, Mn, Fe and Ni, respectively). In addition, variations of only *ca.* ±5% in the H_2O_2 analytical signals in the presence of these concomitants were observed using the spectrophotometric method proposed when these metals were present at the concentrations tested (see Material and Methods).

4.3. Analytical Characteristics and Accuracy Check

After 70 analytical determinations (approximately 40 minutes), the analytical signal for the H_2O_2 standards gave the RSD of 3, 4 and 1%, for SP1, SP2 and SP3 matrices, respectively. In addition, the proposed system was remarkably stable. After 8 h working period, the slope of the calibration curve underwent only slight variations (<2%).

The proposed method covered the 0 – 6.0 mg l^{-1} H_2O_2 concentration range ($r = 0.9902$; $n = 5$) and the detection and quantification limits were 19 and 63 μg l^{-1}, respectively, according to the IUPAC recommendations[22]. These values are much lower than the FDA recommendations, where the H_2O_2 concentration should not be above 0.5 mg l^{-1} in the food or beverage products[23]. Precision, expressed as RSD, was 5% for repeatability and 9% for reproducibility ($n = 9$ for both).

Table 2 shows the levels of H_2O_2 found by two different methods. By applying the *t*-test, the results were found to be similar at the 95% confidence interval, indicating that the proposed method is accurate for these types of samples. In addition, this Table also shows the levels of H_2O_2 found in the water from PET bottles with or without rinse. After the sterilization process, it was possible to observe a reduction in the H_2O_2 concentration from rinsed water bottles. As the H_2O_2 concentration depends on the volume remaining after the rinse process, a wide range of the analytical results for H_2O_2 was obtained. It can be explained either by differences in water stream time during this process or by those in the inner wall bottle surfaces, as already reported by Stannard *et al.*[4].

Table 2 Hydrogen peroxide levels (mg l^{-1}) obtained by automated monosegmented system - MS and the alternative method – AM; n = 3.

Sanificant	With rinse		Without rinse	
	MS	AM	MS	AM
SP1	1.3 – 4.8	1.1 – 4.5	306 – 382	286 – 317
$\bar{x}$	3.1	2.8	336	302
SP2	nd	nd	0.18 – 0.30	0.25 – 0.29
$\bar{x}$	-	-	0.23	0.27
SP3	nd	nd	5.2 – 6.3	5.7 – 6.4
$\bar{x}$	-	-	5.6	5.9

nd = not detected (< 19 μg l^{-1}).

5. Conclusion

The proposed automated monosegmented system for the H_2O_2 determination can be applied in laboratories where monitoring H_2O_2 is required, permitting its determinations at low levels by improving analytical frequency with decrease of sampling handling, time, effort and obtaining accurate results. In addition, it was possible to carry out the H_2O_2 determination using an unstable catalytic reaction for the first time.

It is interesting to point out that the proposed system presented robustness, allowing the performance of 100 determinations per hour, requiring only 0.53 μg of Tiron/determination and reducing drastically the cost per analysis.

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References

[1] K. A. Ito, C. B. Denny, C. K. Brown, M. Yao, M. L. Seeger, *Food Technol.*, **27**, 58, (1973).

[2] D. Raynaud, H. L. M. Lelieveld, *J. Ind. Microbiol. Biot.*, **18**, 326, (1997).

[3] European Hygienic Equipment Design Group (EHEDG). *Trends Food Sci. Tech.*, **4**, 21, (1993).

[4] C. J. Stannard, J. S. Abbiss, J. M. Wood, *J. Food Protect.*, **46**,1060, (1983).

[5] H. B. Chin, A. Cortes, *J. Assoc. Off. Anal. Chem.*, **66**, 199, (1983).

[6] M. Toyoda, Y. Ito, M. Iwaida, M. Fujii, *J. Agric. Food Chem.*, **30**, 346, (1982).

[7] R. M. Sellers, *Analyst*, **105**, 950, (1980).

[8] C. S. Sastry, K. V. S. S. Murty, M. V. Rao, *J. Food Sci. Tech.*, **22**, 420, (1985).

[9] H. Hartkamp, P. Bachhausen, *Atmos. Environ.*, **21**, 2207, (1987).

[10] A. N. Baga, G. R. A. Johnson, N. B. Nazhat, R. A. Saadalla-Nazhat, *Anal. Chim. Acta*, **204**, 349, (1988).

[11] Y. Fujita, I. Mori, M. Toyoda, *Anal. Sci.*, **7**, 327, (1991).

[12] P. A. Clapp, D. F. Evans, *Anal. Chim. Acta*, **243**, 217, (1991).

[13] J. Ruzicka, E. H. Hansen, *Anal. Chim. Acta*, **78**, 145, (1975).

[14] C. Pasquini, W. A. de Oliveira, *Anal. Chem.*, **57**, 2575, (1985).

[15] D. Perez-Bendito, M. Silva, Kinetic Methods in Analytical Chemistry, Ellis Horwood Ltda, Chichester, (1988).

[16] J. Bognár, *Mikrochim. Acta*, **6**, 903, (1961).

[17] M. A. Z. Arruda, E. A. G. Zagatto, A. O. Jacintho, S. M. B. Brienza, *J. Braz. Chem. Soc.*, **2**, 47, (1991).

[18] H. Bergamin-Filho, B. F. Reis, A. O. Jacintho, E. A. G. Zagatto, *Analyst*, **117**, 81, (1980).

[19] H. Afsar, R. Apak and I. Tor, *Analyst*, **115**, 99, (1990).

[20] E. R. Pereira-Filho, M. A. Z. Arruda, *Analyst*, **124**,1873, (1999).

[21] G. E. P. Box, W. G. Hunter, J. S. Hunter, Statistics for experimenters. An introduction to design, data analysis and model building, Wiley, New York, (1978).

[22] Analytical Methods Committee, *Analyst*, **112**, 199, (1987).

[23] H. Reuter, Aseptic Packaging of Food, Technomic, Hamburg, (1993).

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