Sequential injection lab-on-valve system for the on-line monitoring of hydrogen peroxide in lens care solutions

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Keywords:
SI-LOV
Hydrogen peroxide
Horseradish peroxidase
Contact lenses

A B S T R A C T

A sequential injection lab-on valve (SI-LOV) method for the enzymatic determination of hydrogen peroxide was developed. The spectrophotometric assay is based on the reaction between hydrogen peroxide and ABTS (2,2′-Azino-bis(3-Ethylbenzothiazoline 6-sulfonic acid)) in the presence of the enzyme HRP (horseradish peroxidase). The produced oxidized ABTS is measured at 410 nm. The sample consumption was 15 μL/assay and the consumption of HRP and ABTS was 34.6 mg L^{-1} and 0.06 g L^{-1}, respectively with a determination rate of 45 h^{-1}.

Relative deviations lower than 9.0% were found when the results were compared to those obtained by the reference procedure in the analysis of bleaches and disinfection solutions for contact lenses. With the incorporation of an in-line dilution (dialysis) process, was possible to attain a response range up to 342 mg L^{-1} of hydrogen peroxide. The developed method was applied to monitor on-line of the disinfection–neutralization process of contact lenses. The study of two different one-step systems for cleaning contact lenses demonstrated that the neutralization of the hydrogen peroxide is completed within 6 h as recommended by the manufactures. The developed flow method was proved to be a useful tool for monitoring the dynamic process of disinfection–neutralization.

Introduction

Hydrogen peroxide is widespread used in everyday life and in industrial applications due to its oxidative properties. Being a strong oxidative agent it is commonly applied for industrial and household bleaching and it is an ecological friendly option when compared to the chlorine based alternatives. Hydrogen peroxide is also used as powerful antimicrobial agent and effective sporicide [1], and applied in water treatment processes [2], medical instrument sterilization [3] and it can be incorporated into pharmaceutical products [4] for example in the case of disinfections solutions for contact lenses [5,6].

Within the contact lens disinfection processes (thermal and chemical) the hydrogen peroxide based methods show some advantages: it can be applied for repeated disinfection of the lenses, it is more effective against a larger range of microorganisms than the chemical disinfection; and its effervescence also provides mechanical cleaning. However, hydrogen peroxide is toxic to the ocular epithelium and to the cornea, therefore must be neutralized before lens wear [5]. Some of the commercial products perform the neutralization of the hydrogen peroxide right after the disinfection of the lenses, also called one-step system; others, two-step systems, require a neutralization step after the disinfection. In one-step systems the neutralization is achieved in the storage case using a platinum-coated disc (catalytic neutralization) or a soluble catalase tablet (enzymatic neutralization) which catalyses the decomposition of hydrogen peroxide to water and oxygen [5]. The study of the hydrogen peroxide concentration along the disinfection neutralization process is an important way to assess the efficiency and the safety of the procedure. The monitoring can be carried out in batch fashion using titrimetric, spectrophotometric or fluorimetric assays [7], or more efficiently (with lower reagent consumption and higher sample throughput) in flow based methods. Flow based spectrophotometric methods combined with the advantages of highly specific enzyme-based assays could meet the requirements for fast and accurate process monitoring. Most of the spectrophotometric flow methods for the quantification of hydrogen peroxide (Table 1) are based on the use of the peroxidase (EC 1.11.1.7.) enzyme.

In the analytical methodologies that make use of enzymatic reactions, the reduction in the cost of the determination is generally achieved by reducing the reagent consumption. This reduction can be maximised with the miniaturization of the system. Therefore, instead of using the previously mentioned flow injection methodologies (Table 1), for this work the SI-LOV format was selected. In this flow configuration, well-defined volumes of sample and reagents are sequentially aspirated to the holding coil and then propelled by reversed flow towards detection. The miniaturization is feasible due to the proximity of the injection port to the flow cell since in this

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Table 1
Some analytical characteristics of spectrophotometric flow methods for the determination of hydrogen peroxide in different sample matrices.

Flow system	Matrix	λ	Application range	LOD	Reference
Enzymatic FIA	Honey	505 nm	1 to 100 μmol L^{-1}	0.7 μmol L^{-1}	[8]
Enzymatic FIA	Rainwater	505 nm	1 to 100 μmol L^{-1}	0.7 μmol L^{-1}	[9]
Enzymatic FIA	N/A	420 nm	55 to 2730 nmol L^{-1}	N/A	[10]
Enzymatic FIA	N/A	490 nm	Up to 8.8 mmol L^{-1}	0.3 mmol L^{-1}	[11]
FIA, LWCC	Wash solution from cleaning vats in breweries	590 nm	20 to 700 nmol L^{-1}	4 nmol L^{-1}	[1]
FIA	Disinfectants	575 nm	4×10^{-6} to 1×10^{-3} mol L^{-1}	1×10^{-6} mol L^{-1}	[12]
Enzymatic FIA	Pharmaceut.; swimming pool waters	470 nm	1.6×10^{-5} to 6.6×10^{-4} mol L^{-1}	2.1×10^{-6} mol L^{-1}	[4]
Enzymatic FIA	Olive oils and margarines	458 nm	2.5×10^{-5} to 2×10^{-4} mol L^{-1}	0.9 μmol L^{-1}	[13]
FIA	N/A	410 nm	4×10^{-6} to 6×10^{-5} mol L^{-1} [a] 4×10^{-5} to 4×10^{-4} mol L^{-1}	1×10^{-6} mol L^{-1} 2×10^{-5} mol L^{-1}	[14]
FIA	Rain water	450 nm	1×10^{-8} to 1×10^{-5} mol L^{-1}	5×10^{-6} mol L^{-1}	[15]
FIA	Milk	450 nm	10 to 150 mg L^{-1}	N/A	[16]
FIA	Rain water	508 nm	1.36 to 1360 ppb	N/A	[17]
Enzymatic FIA	N/A	414 nm 514 nm 600 nm	0.04 to 200 μmol L^{-1} 0.1 to 500 μmol L^{-1} 0.8 to 1000 μmol L^{-1}	0.1 μmol L^{-1}	[18]
Enzymatic FIA	Rain water	560 nm	1.5×10^{-7} to 4×10^{-5} mol L^{-1}	1.4×10^{-7} mol L^{-1}	[19]

N/A-Not available.
[a]determination with preconcentration.

equipment the manifold/detector is integrated on the top of the selection valve. Under these physical conditions, it is possible to operate at solution volumes as low as a few microliters [20,21]. This flow method has demonstrated to be a promising tool in automating diverse biochemical methods [22–24].

The aim of this work was to develop an enzymatic SI-LOV assay for the determination of hydrogen peroxide in bleaches and in pharmaceutical products and apply it for the on-line monitorization of the disinfection–neutralization process of contact lenses. The quantification of hydrogen peroxide is based on the horseradish peroxidase (HRP) catalysed reaction between H_2O_2 and ABTS [25]. The produced oxidized ABTS is measured at 410 nm. Due to the high concentration of hydrogen peroxide present in the lens care solution, a high dilution step is required. In the present work, this was achieved by incorporating a dialysis unit in which a reduced quantity of the analyte diffuses over the membrane resulting in a high sample dilution.

Experimental

Reagents and solutions

All solutions were analytical grade and deionized water (conductivity <0.1 μS cm^{-1}) was used throughout the work. A potassium phosphate buffer (104873, Merck) (pH 6.0; 100 mmol L^{-1}) solution and an enzyme diluent, containing potassium phosphate buffer (pH 6.8; 40 mmol L^{-1}), 0.25% (w/v) of bovine serum albumin (BSA, fraction V, 05484, Fluka) and 0.5% (v/v) of Triton X-100 (T9284, Sigma), were prepared. Potassium hydroxide (105033, Merck) (1.0 mol L^{-1}) was used to adjust the pH of these buffer solutions.

An enzyme stock solution was prepared by re-suspending 1 mg of lyophilized HRP (Peroxidase from horseradish, EC 1.11.1.7, type VI, P8375, Sigma) in 1.00 mL of enzyme diluent. The working enzyme solution was prepared daily by diluting 90 μL of the stock solution in 2.60 mL of enzyme buffer.

The ABTS stock solution was prepared by dissolving 10 mg (1 tablet) of ABTS (2,2′-Azino-bis(3-Ethylbenzothiazoline 6-sulfonic acid), A 9941,Sigma) in 25.00 mL of deionized water. The daily working solution was prepared by further dilution in potassium phosphate buffer (100 mmol L^{-1}).

The working standard solutions of hydrogen peroxide were prepared from the stock solution (Perhydrol, 30% H_2O_2, 107210, Merck) in a range between 68.4 to 342 mg L^{-1} and 0.54 to 4.28 mg L^{-1}.

Solutions of $Na_2S_2O_3$ (0.1 mol L^{-1}) and $K_2Cr_2O_7$ (0.01 mol L^{-1}), concentrated H_2SO_4, concentrated CH_3COOH; 0.5% (m/v) starch indicator and solid KI were used (all from Merck) to carry out the reference titrimetric method.

Samples

The samples analysed were bleaches and disinfecting solutions for contact lenses containing hydrogen peroxide as an active component. Two brands of bleaches and two kinds of one-step disinfecting solutions for contact lenses (one with enzymatic and the other with catalytic neutralization) were purchased. The samples had no other treatment than dilution, when necessary.

Apparatus

The SI-LOV system (FIAlab-3500, FIAlab Instruments, Medina, WA, USA) presented in Fig. 1, consisting of a bi-directional syringe pump (2500 μL of volume), a holding coil, a bi-directional variable speed peristaltic pump, and a lab-on-valve manifold mounted on the top of a six-port multi-position valve, was used. A laboratory made diffusion unit was incorporated in the manifold. It consisted of two acrylic blocks pressed against each other by four screws with matching cavities of 72 mm^2 of surface area and 0.5 mm of channel depth making a linear path. The in-line dilution was performed by means of a pre-mounted cellulose acetate dialysis membrane. USB 2000 Ocean

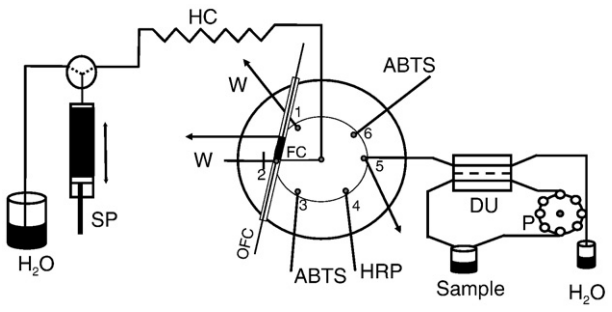

Fig. 1. Configuration of the SI-LOV manifold for the determination of hydrogen peroxide. SP, syringe pump; HC, holding coil; OFC, optical fibre cable; FC, flow cell; ABTS, 0.06 g L^{-1}; HRP, horseradish peroxidase, 34.6 mg L^{-1}; DU, dialysis unit; P, peristaltic pump; W, waste.

Table 2
Flow protocol sequence for both measurements

Step	Description	Volume (μL)	Flow rate (μL s^{-1})	Selection valve position
A	Peristaltic pump on	–	64	–
B	Aspirate carrier to HC	1000	150	–
C	Aspirate ABTS to HC	50	80	3
D	Peristaltic pump off	–	–	–
E	Aspirate sample to HC	15	25	5
F	Aspirate enzyme to HC	15	25	4
G	Aspirate ABTS to HC	100	80	6
H	Reverse flow, reference scan	10	15	2
I	Dispense HC content, data acquisition	400	30	2
J	System washing, SP empty	–	150	2

Optics, a charge coupled device (CCD) array spectrophotometer equipped with fibre optics (id: 200 μm), and a DH-2000-BAL Mikropack, UV/VIS/NIR light source, was used. FIAlab for windows 5.0 software on an Intel Pentium III Computer (995 mHz, 128 MB) was used for flow programming and data acquisition.

Flow procedure

The flow procedure for the determination of hydrogen peroxide using horseradish peroxidase and ABTS is summarised in Table 2.

The initial steps of B–C, and E–G, consisted in the aspiration of carrier, sample, and reagents to the holding coil; (in the order of: 1000 μL of carrier, 50 μL of ABTS, 15 μL of sample, 15 μL of enzyme and 100 μL of ABTS). The following steps (H–J) consisted in reversing the flow and propelling the mixture towards detection for absorbance scanning. The in-line dilution is carried out by means of a dialysis unit incorporated in the system. To execute this in-line dilution it is necessary to include steps A and D to drive the sample towards the dialysis unit and renew the solution at the sampling port while carrier and ABTS are aspirated to the holding coil.

Reference and discussion

The reference method carried out for validation of the results consisted in the iodometric titration of the samples with standardised sodium thiosulphate solution in the presence of potassium iodide and acetic acid [26].

Results and discussions

Study of the floe system

The physical parameters studied were the volumes of the sample and reagent solutions and the aspiration sequence. Initial conditions were established as 0.108 mmol L^{-1} and 23 mg L^{-1} as ABTS and HRP concentrations, respectively, with an aspiration order of: 75 μL of ABTS, 15 μL of enzyme, followed by 15 μL of hydrogen peroxide and 100 μL of ABTS. The ABTS solution was prepared in buffer (pH 6.0); therefore the two plugs of ABTS assured that the reaction occurred at a controlled pH. This optimization study was carried out by performing calibration curves with hydrogen peroxide standards up to 4.28 mg L^{-1}. The sample volume used in the developed method was studied in a range of 15 to 25 μL. Preliminary studies [27] indicated

Table 3
Configuration of the mass transfer units studied

Mass transfer unit	Configuration	Surface area (mm^2)	Channel depth (mm)
A	Linear	72	0.5
B	Linear	140	0.5
C	Zig-zag	146	0.3

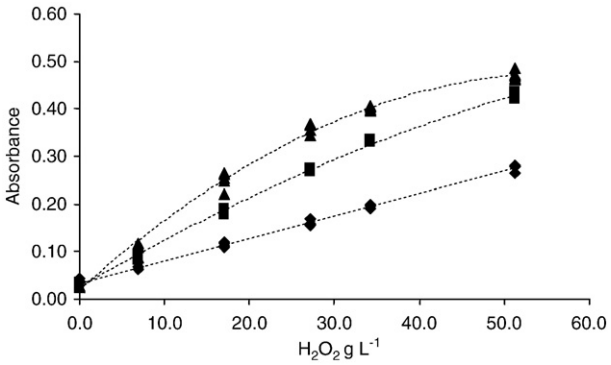

Fig. 2. Calibration curves obtained using different mass transfer unit configurations: ◆ Unit A; ■ Unit B; ▲ Unit C. Other conditions: HVPV DURAPORE membrane filter; 0.06 g L^{-1} of ABTS; 34.6 mg L^{-1} of enzyme; hydrogen peroxide standards up to 51.3 g L^{-1}.

that lower volumes would deteriorate the repeatability of the method. The highest sensitivity was obtained with the volume of 15 μL, as it was possible to achieve a better overlapping of the reagent zones involved in the reaction [27]. For that reason this was the volume used for the developed method as a good compromise between sensitivity and repeatability.

The enzyme solution volume was studied in a range from 5 to 15 μL. A 15% increase in the sensitivity was achieved by the increase of enzyme solution volume from 5 to 15 μL; therefore the chosen volume for the developed method was 15 μL. The volume of ABTS used in the rear part of the aspiration sequence was also studied (50, 75 and 100 μL). When the assay was performed with 50 μL higher sensitivity was obtained and with the increase to 75 μL of ABTS a decrease in sensitivity about 8% was found; therefore the volume of 50 μL was chosen. Additionally the aspiration sequence was studied by altering the order of sample and enzyme aspiration. Calibration curves were established using standards of hydrogen peroxide in a range of 1.19 to 4.76 mg L^{-1}. When sample was aspirated after the enzyme solution, the results showed higher analytical signal; but lower repeatability. As there were no significant differences in the sensitivity between the cases studied, the selected sequence of aspiration for the developed assay was: ABTS–sample-enzyme–ABTS.

The flow rate used to propel the product of the reaction towards detection was studied with the purpose of improving the sample throughput of the assay. For this study a standard of 137 mg L^{-1} of hydrogen peroxide was analysed and flow rates in the range from 15 to 60 μL s^{-1} were tested. The results were evaluated in terms of repeatability and in terms of the analytical signal obtained. The value of the analytical signal obtained for all of the cases revealed no significant differences but the repeatability deteriorated for the flow rate of 60 μL s^{-1}. An intermediate flow rate (30 μL s^{-1}) was chosen for

Table 4
Figures of merit of the developed methods

Parameter	Value
Reagent consumption/assay	
ABTS	9.0 μg
HRP	0.52 μg
Sample	15 μL
Waste production/assay	1.2 mL
Determination rate	45 h^{-1}
Application range	Up to 4.28 mg L^{-1} [a] Up to 342 mg L^{-1} [b]
LOD	0.20 mg L^{1} [a] 16.1 mg L^{-1} [b]
LOQ	0.45 mg L^{1} [a] 44.3 mg L^{-1} [b]

[a] Measurement of hydrogen peroxide with off-line dilution.
[b] Measurement of hydrogen peroxide with in-line dilution.

Table 5
Results obtained for the analysis of different samples

Sample	Reference method (g L^{-1})[a]	Developed method (g L^{-1})[b]	R.D.[c]%
Bleach 1	71.3±0.3	70.2[d]±2.5	−1.3
Bleach 2	84.4±0.5	91.8[d]±4.2	8.9
Lens care solution 1, catalytic neutralization	36.4±0.3	36.6[e]±0.8	0.5
Lens care solution 2, enzymatic neutralization	37.0±0.4	36.3[e]±1.8	−1.9

[a] mean and standard deviation for $n=3$.
[b] mean and standard deviation for $n=5$.
[c] Relative deviation.
[d] with off-line dilution.
[e] with in-line dilution.

further work as good repeatability (RSD<3%; $n=9$) was achieved under these conditions.

After establishing the physical conditions of the system, the effect of ABTS and enzyme concentration on the analytical signal was also studied to ensure that their concentrations were not the limiting one in this application range. Calibrations curves up to 4.28 mg L^{-1} of hydrogen peroxide were performed for the study. The concentration of ABTS was studied in the range of 0.047 to 0.20 g L^{-1}. For concentrations higher than 0.06 g L^{-1} of ABTS no significant increase on the sensitivity was achieved, therefore this concentration was chosen. The enzyme concentration was studied in a range between 23 to 46 mg L^{-1}. At concentrations higher than 34.6 mg L^{-1} there was no significant increase in the sensitivity (1.1%), thus this concentration was chosen for further work.

The developed method presented a sample consumption of 15 μL/assay and a consumption of enzyme and ABTS of 34.6 mg L^{-1} and 0.06 g L^{-1}, respectively. The determination rate was 45 h^{-1}, and was possible to achieve a determination range up to 4.28 mg L^{-1}. The limits of quantification and determination [28] obtained were 0.45 and 0.20 mg L^{-1}, respectively.

Due to the elevated concentration of hydrogen peroxide present in the cleaning solutions, a high dilution of the samples is required. To carry out the in-line dilution, a mass transfer unit was introduced in the system, where the analyte diffuses across the membrane from the donor channel to the acceptor stream, resulting in the dilution of the sample. Three mass transfer units with different configurations (Table 3) were evaluated.

To evaluate the performance of the mass transfer units, a hydrophobic DURAPORE membrane filter (HVPV, 0.45 μm) was used and calibration curves were carried out with hydrogen peroxide standards in a range up to 51.3 g L^{-1}, as presented in Fig. 2.

As expected, the sensitivity increased with the increase of the surface area of the units; therefore the lowest sensitivity was found using unit A. The chosen unit for the work was unit A for presenting a wider linear range with higher sample dilution.

Hydrophobic and hydrophilic membranes were tested to perform the in-line dilution. The hydrophobic membranes were not compatible with the samples. After contact with the sample matrix the analytical signal obtained was constantly increasing, that can be explained by the presence of surface active substances in the sample that affect the hydrophobicity of the membrane. When hydrophilic dialysis membranes were used, this behaviour was not noticed.

Two different types of hydrophilic membranes were tested, cellophane and pre-mounted cellulose acetate dialysis membrane. With the use of the latter membrane the sensitivity increased about 65%, (while the linear response range was maintained in the same extent); therefore the pre-mounted membrane was chosen for performing the in-line dilution of the developed method. With the use of the selected membrane it was possible to achieve an in-line dilution of approximately 80 times.

Figures of merit of the methods

The developed method with in line dilution was re-evaluated in terms of reagent consumption; determination rate and application range and was compared with the system without in-line dilution (Table 4). The reagent consumption was maintained as in the previous system; however it was possible to achieve a determination range up to 342 mg L^{-1} with a limit of quantification of 44.3 mg L^{-1}, and a limit of detection of 16.1 mg L^{-1}.

Sample analysis

Two different types of samples (two brands of bleaches and lens care solutions) were used to evaluate the accuracy of the developed methods. The values obtained for all samples were in agreement with those obtained by the reference method, as it can be concluded from the RD values achieved, as reported in Table 5.

On-line monitoring of the neutralisation of the contact lenses desinfection/neutralization process

In this work, the neutralization process of two different one-step systems, catalytic and enzymatic (Fig. 3), were evaluated. The analytical signal resultant of the presence of hydrogen peroxide during the disinfection–neutralization process was recorded. Initial concentration of hydrogen peroxide in the disinfecting solution

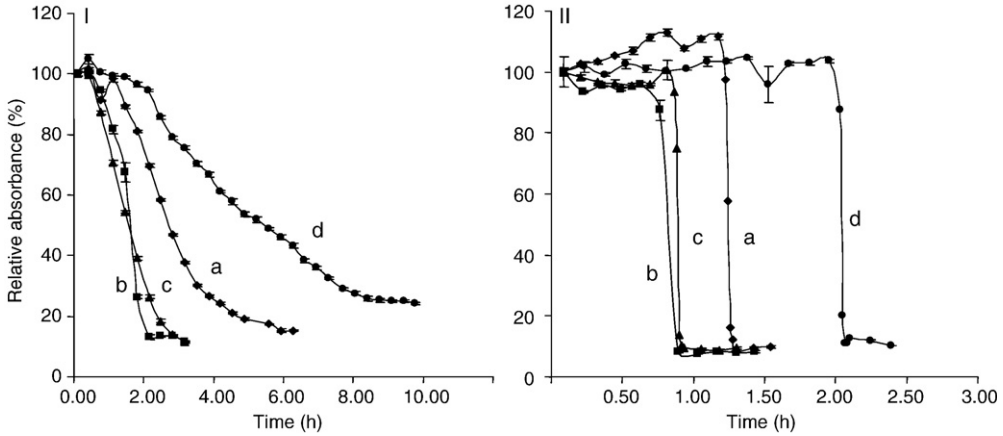

Fig. 3. Relative absorbance of the hydrogen peroxide disinfection–neutralization process for sample I (catalytic neutralization) and for sample II (enzymatic neutralization) in different conditions: a recommended; b with mechanical stirring; c at 30 °C; d at 2 °C. Other conditions: 0.06 g L^{-1} of ABTS; 34.6 mg L^{-1} of enzyme.

situated over the upper limit of the linear response range of the method. Therefore, an option was taken to present the results as a percentage of degradation of the hydrogen peroxide, relative to the recorded initial absorbance.

Performing the disinfection–neutralization process with catalytic neutralization (Fig. 3I) as recommended by the manufacturer and described in the label of the product (Fig. 3I, curve a) it can be concluded that the total neutralization is achieved in almost 6 h. When the process is performed with mechanical stirring (Fig. 3Ib), the neutralization is achieved in around 2 h; this is also the time necessary to attain the neutralization at 30 °C (Fig. 3Ic). When the process occurs at 2 °C (Fig. 3Id) it is necessary to wait almost 8 h to obtain the neutralization of the hydrogen peroxide.

When the process occurs with enzymatic neutralization (catalase, Fig. 3II), the total neutralization of hydrogen peroxide is achieved faster than the same procedure with catalytic neutralization. When the process is performed as recommended by the manufacturer (Fig. 3IIa) it is not necessary wait more than one and half hour to achieve the neutralization [6]. After the disinfection time, about 1 h, the catalase is released from the nucleus of the tablet and the neutralization process starts promptly. When the assay is performed with mechanical stirring (Fig. 3IIb) or at 30 °C (Fig. 3IIc) the total neutralization is achieved in less than 1 h; and in a little more than 2 h the neutralization is complete if the process is performed at 2 °C (Fig. 3IId). The neutralization profile obtained for this process is very different from the one obtained for the process with catalytic neutralization.

Conclusions

The developed method proved to be a useful tool for the monitoring of the disinfection/neutralization process for the cleaning of contact lenses; the applied configuration is compact and miniaturized, and makes use of commercially available reagents. With the introduction of a dialysis unit in the system, it was possible to achieve a high in-line dilution factor of the sample, making the monitoring of this dynamic process possible.

The advantages of flow dialysis processes for analyte separation and/or sample dilution have been widely exploited since the first time applied in flow injection analysis by Ruzicka and Hansen [29] and in sequential injection by van Staden [30]. However, to the best of our knowledge, this is the first time that the dialysis process is used to perform the in-line dilution of the sample in the sequential injection lab-on-valve format.

Acknowledgements

Susana Vidigal and Ildikó Tóth thank Fundação para a Ciência e a Tecnologia (FCT) and FSE (III Quadro Comunitário) for the grants SFRH/BD/23040/2005 and SFRH/BPD/5631/2001, respectively.

References

[1] A.C. Pappas, C.D. Stalikas, Y.C. Fiamegos, M.I. Karayannis, Determination of hydrogen peroxide by using a flow injection system with immobilized peroxide and long path length capillary spectrophotometry, Anal. Chim. Acta 455 (2002) 305–313.
[2] K. Ikehata, M. Gamal El-Din, Aqueous pesticide degradation by hydrogen peroxide/ultraviolet irradiation and Fenton-type advanced oxidation processes: a review, J. Environ. Eng. Sci. 5 (2006) 81–135.
[3] F. Guillaume, E. Comoy, C. Duval, K. Antloga, C. Dehen, A. Charbonnier, G. McDonnell, C.I. Lasmézas, J.P. Delys, Novel methods for disinfection of prion-contaminated medical devices, Lancet 364 (2004) 521–526.
[4] I.C. Vieira, O. Fatibello-Filho, Flow injection spectrophotometric determination of hydrogen peroxide using a crude extract of zucchini (*Cucurbita pepo*) as a source of peroxidase, Analyst 123 (1998) 1809–1812.
[5] S. Kilvington, Antimocrobial efficacy of a povidone iodine (PI) and a one-step hydrogen peroxide contact lens disinfection system, Cont. Lens Anterior Eye 27 (2004) 209–212.
[6] K. Hiti, J. Walochnik, C. Faschinger, E.M. Haller-Schober, H. Aspöck, One- and two-step hydrogen peroxide contact lens disinfection solutions against *Acanthamoeba*how effective are they? Eye 19 (2005) 1301–1305.
[7] I.L. Mattos, K.A. Shiraishi, A.D. Braz, J.R. Fernandes, Peróxido de hidrogénio: importância e determinação, Quim Nova 26 (2003) 373–380.
[8] R.A.A. Franchini, C.F. Souza, R. Colombara, M.A.C. Matos, R.C. Matos, Rapid determination of hydrogen peroxide using immobilized on Amberlite IRA-743 and minerals in honey, J. Agric. Food Chem. 55 (2007) 6885–6890.
[9] R.C. Matos, E.O. Coelho, C.F. Souza, F.A. Guedes, M.A.C. Matos, Peroxidase immobilized on Amberlite IRA-743 resin for on-line spectrophotometric detection of hydrogen peroxide in rainwater, Talanta 69 (2006) 1208–1214.
[10] K.F. Fernandes, C.S. Lima, F.M. Lopes, C.H. Collins, Hydrogen peroxide detection system consisting of chemically immobilised peroxidase and spectrometer, Process Biochem. 40 (2005) 3441–3445.
[11] V. Vojinović, A.M. Azevedo, V.C.B. Martins, J.M.S. Cabral, T.D. Gibson, L.P. Fonseca, Assay of H_2O_2 by HRP catalysed co-oxidation of phenol-4-sulphonic acid and 4-aminoantipyrine: characterisation and optimisation, J. Mol. Catal. B-Enzym 28 (2004) 129–135.
[12] D. Harms, R. Than, B. Krebs, U. Karst, Flow injection analysis of hydrogen peroxide in disinfectants, Fresenius J. Anal. Chem. 364 (1999) 184–188.
[13] G.P. Avila, A. Salvador, M. de la Guardia, Enzymatic determination of peroxides in non-aqueous media, Analyst 122 (1997) 1543–1547.
[14] A.M. Almuaibed, A. Townshend, Flow spectrophotometric method for determination hydrogen peroxide using a cation exchanger for preconcentration, Anal. Chim. Acta 295 (1994) 159–163.
[15] C. Matsubara, N. Nakamichi, N. Kawamoto, K. Takamura, Flow-injection analysis for hydrogen-peroxide using a Titanium(IV)-Porphirin complex, Bunseki Kagaku 42 (1993) 363–367.
[16] J.F. Cerdán, M. Peris-Tortajada, R. Puchades, A. Maquieira, Automation of the determination of hydrogen peroxide, dichromate, formaldehyde and bicarbonate in milk by flow injection analysis, Fresenius J. Anal. Chem. 344 (1992) 123–127.
[17] C. Matsubara, K. Sakai, K. Takamura, Determination of hydrogen-peroxide in rainwater by flow-injection analysis with Titanium(IV)-4-(2-Pyridylazo)Resorcinol reagent, Nippon Kagaku Kaishi 5 (1991) 430–432.
[18] B. Olsson, A flow-injection system using immobilized peroxidase and chromogenic reagents for possible determination of hydrogen peroxide, Microchim. Acta 86 (1985) 211–221.
[19] B.C. Madsen, M.S. Kromis, Flow injection and photometric determination of hydrogen peroxide in rainwater with N-Ethil-N-(sulfoproly)aniline sodium salt, Anal. Chem. 56 (1984) 2849–2850.
[20] J. Wang, E.H. Hansen, Sequential injection lab-on-valve: the third generation of flow injection analysis, Trends Anal. Chem. 22 (2003) 225–231.
[21] J. Ruzicka, Lab-on-valve: universal microflow analyser based on sequential and bead injection, Analyst 125 (2000) 1053–1060.
[22] M.D. Luque de Castro, J. Ruiz-Jiménez, J.A. Pérez-Serradilla, Lab-on-valve: a useful tool in biochemical analysis, Trends Anal. Chem. 27 (2008) 118–126.
[23] E.H. Hansen, M. Miró, How flow-injection analysis (FIA) over the past 25 years has changed our way of performing chemical analysis, Trends Anal. Chem. 26 (2007) 18–26.
[24] X.W. Chen, J.H. Wang, The miniaturisation of bioanalytical assays and sample pretreatments by exploiting meso-flluidic lab-on-valve configurations: a review, Anal. Chim. Acta 602 (2007) 173–180.
[25] G. Durand, P. Monsan, Les enzymes: production et utilisations industrielles, Gauthier-Villars, Paris, 1992, pp. 199–258.
[26] APHA-AWWA-WPCF, Standard methods for the examination of water and wastewater, American Public Health Association, Washington, DC20th ed., 1998, pp. 4.55–4.56.
[27] S.S.M.P. Vidigal, I.V. Tóth, A.O.S.S. Rangel, Sequential injection-LOV format for peak height and kinetic measurements modes in the spectrophotometric enzymatic determination of ethanol: application to different alcoholic beverages, Talanta 77 (2008) 494–499.
[28] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed, Ellis Horwood, New York, 1993, pp. 101–139.
[29] E.H. Hansen, J. Ruzicka, Flow injection analysis Part VI. The determination of phosphate and chloride in blood serum by dialysis and sample dilution, Anal. Chim. Acta 87 (1976) 353–363.
[30] J.F. van Staden, H. du Plessis, R.E. Taljaard, Determiantion of iron (III) in pharmaceutical samples using dialysis in a sequential injection analysis system, Anal. Chim. Acta, 357 (1997) 141–149.