Near-Infrared (NIR) Monitoring of H_2O_2 Vapor Concentration During Vapor Hydrogen Peroxide (VHP) Sterilisation

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Purpose. There is an increasing use in the pharmaceutical industry of barrier systems such as transfer isolators, sterilisation tunnels and work station isolators. As Vapor Hydrogen Peroxide (VHP) sterilisation of isolators and lyophilizers becomes an important sterilisation method, there is an acute need for a VHP monitoring system to be used for in-process control and validation. In this study, near infrared (NIR) spectrofotometry was evaluated as a potential technique to monitor hydrogen peroxide. Additionally the H_2O_2 vapor permeability of different packaging materials, commonly used in steam and ethylene oxide sterilisation, was evaluated.

Methods. NIR spectrofotometry, using a gas cell connected with optic fibres, was evaluated as a potential technique to monitor hydrogen peroxide vapor and water vapor during VHP sterilisation of an isolator. A NIR spectrum was taken every 30 s during VHP sterilisation of an isolator. The influence of injection rate, air flow rate, working temperature and gas distribution was investigated. The H_2O_2 vapor permeability of different packaging materials was determined by placing the gas cell in the sterilisation bags and sealing the bags hermetically. The sterilisation bag was then subjected to VHP sterilisation.

Results. The NIR spectra taken at steady state sterilization conditions showed 4 absorption peaks: at 1364,1378 and 1400 nm attributed to water and at 1420 nm attributed to H_2O_2 vapor. By measuring the absorbance level at these wavelengths, the actual concentration of H_2O and H_2O_2 vapor in the isolator was calculated. The water vapor permeation of the sterilisation bags, measured with NIR, appeared to be equal for all materials tested. Whereas Tyvek® was the most permeable material for hydrogen peroxide vapor (82.7% of the reference concentration outside the bag), only 30% was found in bags made of medical paper. Sterilisation bags consisting of laminate films and PVC sealed to medical paper showed intermediate permeability.

Conclusions. Near-infrared (NIR) spectroscopy using a gascell with optic fibres is a useful technique to monitor VHP sterilisation cycles. There was a difference in H_2O_2 vapor permeability of different packaging materials, commonly used in steam and ethylene oxide sterilisation.

KEY WORDS: hydrogen peroxide monitoring; near-infrared; VHP sterilisation.

INTRODUCTION

There is an increasing use in the pharmaceutical industry of barrier systems such as transfer isolators, sterilisation tunnels and work station isolators. These isolators are designed to be used for sterility testing, for batch and continuous production and to perform aseptic manipulations (1). The methods used to sterilize the interior of these isolators have to be effective and minimize or eliminate toxic residue concerns or materials compatibility problems. Vapor hydrogen peroxide (VHP) is a cold gas sterilant (2), which is a good alternative for previously used decontamination methods, such as ethylene oxide, formaldehyde and peracetic acid (3). Hydrogen peroxide vapor sterilisation has shown to be an effective biodecontamination method for a wide range of microorganisms (4). Previous studies revealed Bacillus stearothermophyllus as the most resistant organism to VHP (5). Vaporised hydrogen peroxide (VHP) can be used in the biodecontamination of isolators (6,7) and lyophilizers (8). As VHP sterilisation of isolators becomes an important sterilisation method, there is an acute need for a VHP gas monitoring system that can be used for both in-process control and validation. In this study a near-infrared (NIR) spectofotometer using a gas cell connected with optic fibres was evaluated as a potential technique to monitor hydrogen peroxide vapor and water vapor during VHP sterilisation. The H_2O_2 vapor permeability of different packaging materials, commonly used in steam or ethylene oxide sterilisation was evaluated.

MATERIALS AND METHODS

Hydrogen Peroxide Solution

The H_2O_2 solution used during sterilisation theoretically contained 31% (w/w) H_2O_2 (lotPE074C) and was obtained from Amsco (Apex, NC, USA).

Sterilisation Cycles

A VHP 1001 Generator (Amsco, Apex, NC, USA) was used to deliver hydrogen peroxide vapor to a 1.024 m^3 flexible wall work isolator Model 2003 and a 0.51 m^3 transfer isolator manufactured by La Calhène (Velizy Cedex, France) in a closed loop configuration. The VHP inlet port was positioned between the isolator blower and the HEPA filter. The sterilant gas was blown through the HEPA filter into the transfer isolator. The peroxide vapor outlet was directed through another HEPA filter. During all the experiments, the isolators were in a not loaded configuration. The programmed VHP sterilisation cycles consisted of three major steps:

1. Dehumidification: the isolator was dehumidified to 10.0% RH (relative humidity) to remove water vapor in order to prevent hydrogen peroxide vapor condensation. The minimum programmed dehumidification time was 10 min at 34 m^3/h.
2. Sterilisation: the generator was programmed to deliver different amounts (1–3 g/min) of the hydrogen peroxide solution per minute and the air flow was varied between 17 and 34 m^3/h. The total sterilisation time was kept constant at 30 min.
3. Aeration: after sterilisation, the isolator was aerated for 60 min at different air flow rates ranging from 17 m^3/h to 34 m^3/h.

An overview of all the sterilisation parameters during the experiments is given in Table I. Fans were used in this study to improve gas distribution (Etri, model 125 XR). In the transfer isolator a fan was located at the centre of the isolator floor and in the work isolator the 3 fans were located at 3 different sites:

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Table I. Sterilization Parameters During the NIR Evaluation Experiments

Isolator	H_2O_2 injection rate	Air flow rate (sterilization)
Transfer 0.51 m^3 (1 fan)	1.5 g/min	22 m^3/h
	1.8 g/min	22 m^3/h
	2.5 g/min	22 m^3/h
Workisolator 1.024 m^3 (3 fans)	2.6 g/min	17 m^3/h[a]
		22 m^3/h[a]
		34 m^3/h[a]

[a] Experiments both at 25°C and at 32°C.

2 on the upper side walls of the isolator blowing towards the middle of the isolator floor, and one in the middle of the isolator floor blowing upwards. The Chemdi-VHP short strip (Amsco, Apex, NC, USA) was used as chemical indicator (CI). This CI is a 1.5 cm × 5 cm paper strip with a yellow ink indicator responsive to hydrogen peroxide vapor. The yellow color changes to violet-grey following hydrogen peroxide gas exposure. A total of 12 CI were used for each sterilisation cycle. The CI's were positioned in the 8 corners of the isolator and on the 4 PVC walls.

Dräger tubes (Dräger, Lubeck, Germany) were used to evaluate the hydrogen peroxide concentration in the low concentration range during aeration.

Near-Infrared (NIR) Analyser

Near-infrared spectroscopy is used to measure the absorbance in the 1200–1600 nm range using a ProSpec® IV spectrophotometer (UOP/Guided-Wave®, Antwerpen, Belgium). The NIR energy is transmitted to the sampling point in the isolator by a low-OH silica fiber optic cable (UOP/Guided-Wave®, Antwerpen, Belgium). The gas sampling cell consisted of a 20 cm length by 12 mm diameter-316 stainless steel housing with 2 lenses of Bk7 glass. NIR spectra were taken every 30 s during all VHP cycles. The H_2O_2 and H_2O vapor concentrations were calculated by an IBM compatible computer, connected to and controlling the ProSpec® IV spectrophotometer.

Three individual calibration curves were plotted on three different days, between the theoretical or calculated H_2O_2 vapor concentration (x-values) and the levels determined at steady state (y-values). The three theoretical concentrations chosen were: 604, 887 and 1293 ppm. At each steady state level, 13 determinations of the H_2O_2 vapor concentration were performed. The y-points of the calibration curves were taken as the average value of these 13 determinations. The calibration curves showed a linear correlation with the following characteristics: slope 0.9467 (5% C.I. 0.8894–1.0041), intercept 131.6717 (5% C.I. 74.0749–189.2689) and an r^2 value of 0.969736 for the first calibration curve, slope 0.8929 (5% C.I. 0.8273–0.9585), intercept 184.7765 (5% C.I. 118.92–250.62) and an r^2 value of 0.9561 for the second calibration curve and a slope of 1.1088 (5% C.I. 0.8271–1.3906), intercept 27.5256 (5% C.I. 1.7622–56.8133) and an r^2-value of 0.997013 for the third calibration curve.

Packaging Materials

The following packaging materials were used: two different medical paper sterilisation bags were used in this study: SBW medical paper (Griffith microscience, Herentals, Belgium) and SPS medical paper (SPS Laboratoires, Coulommiers, France); spunbonded polyethylene Tyvek® L1073B (Dupont, Luxemburg) and sterilizable RfS® bags, Tyvek L1073B sealed to a high density polyethylene film (Helvoet Pharma, Alken, Belgium); Teijen T85393, woven Tafeta 1/1 (Countdown, Wilrijk, Belgium); SPS Pealpack®, a polyester-polypropylene film sealed to a medical paper backing (SPS Laboratoires, Coulommiers, France); View-pack, a transparant blue lamination of polyester and polypropylene film, sealed to a paper backing (SBW, LMG Smith Brothers, Whitehaven, UK); sterilisation bags of polyethylene-polyamide laminate sealed to medical paper and boxes of PVC sealed to a paper back (Sterima, Bissegem, Belgium). The H_2O_2 vapor measuring cell was placed in the sterilisation bags and the bags were sealed hermetically, next the packaging material was subjected to sterilisation as described previously.

RESULTS AND DISCUSSION

Evaluation of the NIR Technique to Monitor VHP Sterilisation

Near-infrared (NIR) spectroscopy has been extensively used in the food industry for the past twenty years (9) and became an important technique in the pharmaceutical industry in recent years (10). NIR spectroscopy has been reported as a technique to determine the water content of freeze-dried products (11), and a rapid method for the identification of active components in tablets (12) and liquid formulations (13). NIR spectroscopy has also been evaluated as a potential monitoring system in the film coating process (14). NIR spectroscopy can be used to monitor VHP sterilisation, since the two major gas components, hydrogen peroxide vapor and water vapor, absorb NIR light at different wavelengths.

NIR spectra taken during different phases of the sterilisation cycle of a transfer isolator are shown in Fig 1. Spectrum A, taken at steady state sterilisation conditions, shows 4 absorption peaks in the NIR region. The peaks at 1364 nm, 1378 nm and 1400 nm, respectively are to be attributed to water. The absorption peak at 1420 nm is assigned to H_2O_2. The water vapor content of the isolator is determined by measuring the absorbance at 1364 nm, 1378 nm and 1400 nm. By measuring the absorbance at 1420 nm, the actual concentration of H_2O_2 vapor in the isolator was determined. Spectra B and C were taken during the aeration phase after 10 and 20 min, respectively. The absorption at 1364 nm, 1378 nm and 1400 nm, attributed to the water vapor decreased faster compared to the H_2O_2 vapor absorption. H_2O_2 vapor concentration was also monitored during sterilisation of the transfer isolator at different H_2O_2 injection rates (1.5, 1.8 and 2.5 g/min) and at a constant air flow rate of 22 m^3/h. The theoretical H_2O_2 concentrations inside the isolator during these sterilisation cycles were calculated according to the method described by Amsco (15). The correlation between the NIR measured concentrations and the calculated concentrations is seen in Table II. There was a linear correlation between theoretical concentration and the actual concentration measured with NIR.

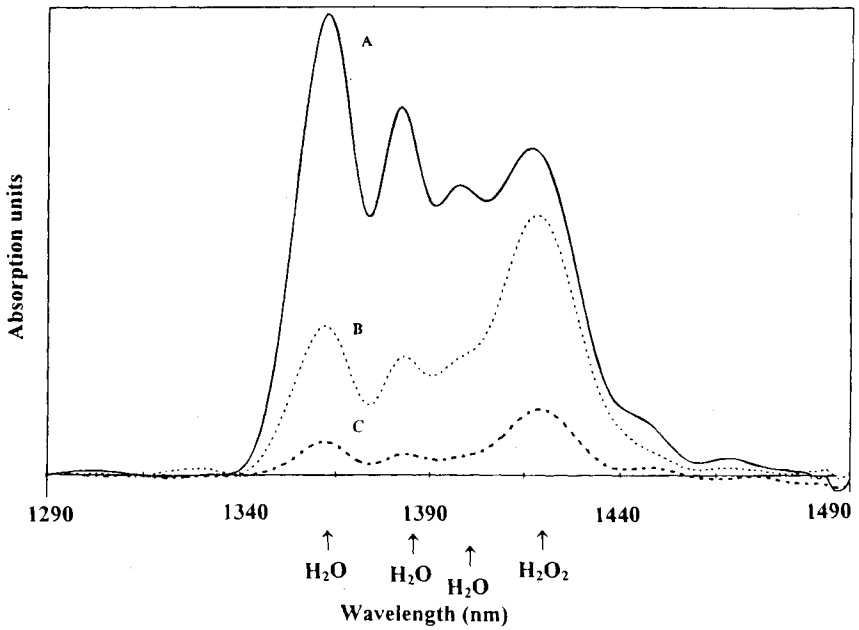

Fig. 1. NIR spectra taken during different phases of a VHP sterilization cycle of transferisolator: at steady state sterilization conditions (A), after 10 min aeration (B) and after 20 min aeration (C).

Table II. Theoretical (Calculated) H_2O_2 Vapor Concentrations vs NIR Measurements During VHP Sterilisation of a Work Isolator Using an Air Flow Rate of 34 m^3/h

H_2O_2 injection rate (g/min)	Theoretical H_2O_2 vapor conc (ppm)	NIR measured H_2O_2 vapor conc (ppm)
1.3	511	602
2	714	850
2.5	819	920

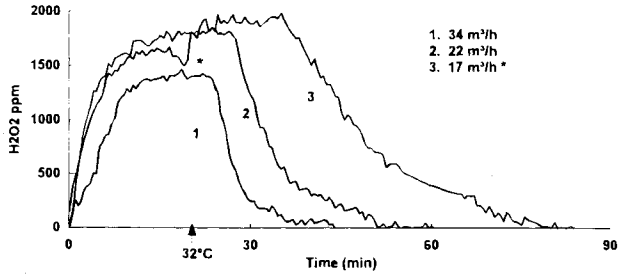

Fig. 2. Hydrogen peroxide vapor concentration during VHP sterilization of a workisolator at 25°C, using different H_2O_2 air flow rates: 34 m^3/h (1), 22 m^3/h (2) and 17 m^3/h (3). *Temperature in the isolator was raised to 32°C.

The water vapor (mg/L) and hydrogen peroxide vapor concentrations (ppm) were determined during sterilisation of a 1.024 m^3 work isolator at an injection rate of 2.6 g/min, at air flow rates of 17, 22 and 34 m^3/h, respectively and at temperatures of 25 and 32°C (Fig 2). Three fans were used in the work isolator and were located as described previously to improve the gas distribution. At air flow rates of 34 m^3/h and 22 m^3/h, a steady state H_2O_2 vapor concentration of 1370 ppm and 1703 ppm, respectively, was measured at a temperature of 25 ± 1.5°C. These steady state concentrations were reached within 10 min, which correlated well with the chemical indicator (CI) colour change. All the 12 CI started to change colour within 3–5 min and were completely violet-grey within 10–12 min. At an air flow rate of 17 m^3/h, the H_2O_2 vapor concentration increased to 1600 ppm within 10 min, but then started to decrease (Figure 2), when condensation occurred on the PVC walls of the isolator. During this sterilisation cycle, we decided to raise the temperature inside the isolator to 32 ± 1.8°C and immediately the H_2O_2 concentration increased to 1985 ppm. In a second experiment, the temperature inside the isolator was adjusted to 32°C. Next the sterilisation cycles were started, using an injection rate of 2.6 g/min and air flow rates of 17, 32 and 34 m^3/h, respectively. During these experiments at 32°C, no condensation occured on the PVC walls of the isolator. The steady state H_2O_2 vapor concentration at 34 m^3/h and 22 m^3/h were 1504 and 2099 ppm, respectively. These concentrations were higher in comparison with the same experiments at 25°C (1420 and 1750 ppm, using 34 and 22 m^3/h, respectively). At the air flow rate of 17 m^3/h the maximum H_2O_2 vapor concentration was 2095 ppm.

During aeration the hydrogen peroxide vapor concentration decreased in two steps. When the steady state H_2O_2 vapor concentration of 1550 ppm was reached, the isolator was aerated to a H_2O_2 level below 50 ppm in 33 min and in 50 min (measured with NIR), at air flow rates of 34 m^3/h and 17 m^3/h, respectively. The hydrogen peroxide vapor concentration could not be accurately measured during the second aeration step, because of the limited sensitivity of the NIR measuring cell. Using Drager tubes to determine the H_2O_2 gas concentration below 3 ppm, a level of 0.1 ppm was reached in 75 min and in 123 min at an air flow rate of 34 m^3/h and 17 m^3/h, respectively.

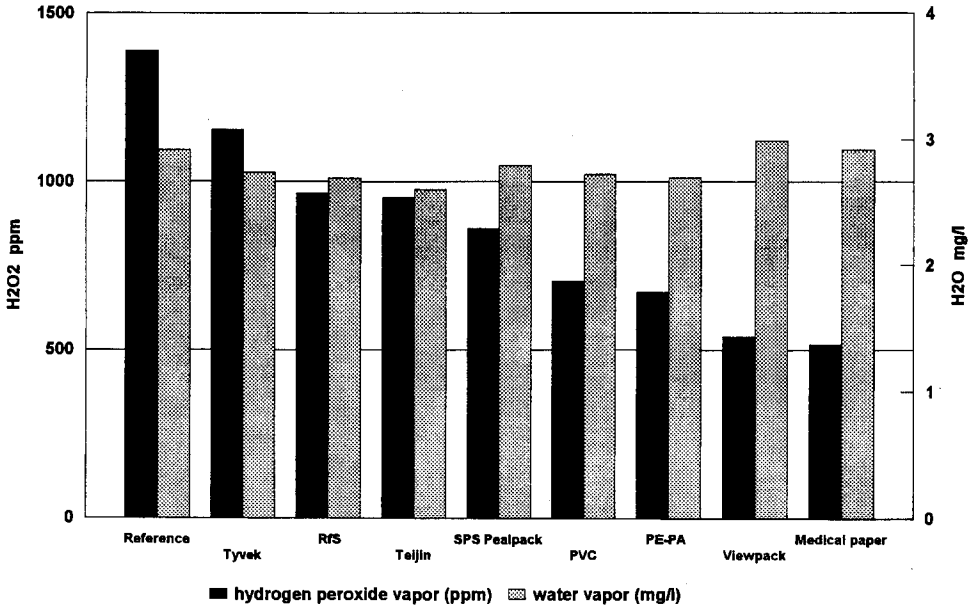

Fig. 3. Average concentrations between 20 and 30 min during VHP sterilization, inside the transferisolator (reference) and in different sterilization bags: hydrogen peroxide vapor (ppm); water vapor (mg/L).

Hydrogen Peroxide Permeation of Different Packaging Materials

The experiments were performed in the 0.51 m^3 transferisolator, using a VHP injection rate of 1.5 g/min at an air flow rate of 22 m^3/h. The water vapor concentration (mg/L) and H_2O_2 vapor concentration (ppm) inside the transferisolator and sterilisation bags, after 20–30 min sterilisation time, are shown in Fig 3. The water vapor permeation, measured with NIR appeared to be equal for all materials tested. The maximal water vapor concentration was in the range 2.6–2.9 mg/L. This is not unexpected, since all sterilisation bags are used for steam sterilisation. Tyvek® appeared to be the most permeable material for hydrogen peroxide vapor. A concentration of 1204 ± 26 ppm H_2O_2 was measured inside the Tyvek® bag, being 82.7% of the reference concentration outside the bag. Tyvek® consists of a porous network, which allows gas sterilisation and was reported to be permeable to ethylene oxide gas. The average gas permeability of the RfS® bags, consisting of Tyvek® sealed to high density polyethylene and the Teijin which is a 1/1 Tafeta woven material was 67.4% and 66.3% of the reference, respectively. The bags consisting of laminate films (eg. polyethylene-polyamide, polyester to polypropylene, etc. . . .) and PVC sealed to medical paper appeared to be less permeable to H_2O_2 vapor (42.%–56.7% of the reference). The gas penetration of the medical paper was 30%. A concentration-time profile of hydrogen peroxide vapor inside Tyvek®, SPS Pealpack® and medical paper bags, using the same sterilisation conditions, is shown in Fig 4. There was a great difference in the hydrogen peroxide vapor concentration inside the sterilisation bags, although the water vapor concentration was similar for all the materials tested. It is very unlikely that a difference in porosity between the materials caused these differences, especially since the water vapor concentration was similar in all the bags. The difference in H_2O_2 vapor concentration could be due to an absorption phenomenon or to a difference in H_2O_2 degradation rate caused by the presence of trace metals in the packaging materials, acting as a catalyst for the hydrogen peroxide breakdown, both parameters being function of the composition of the bag materials.

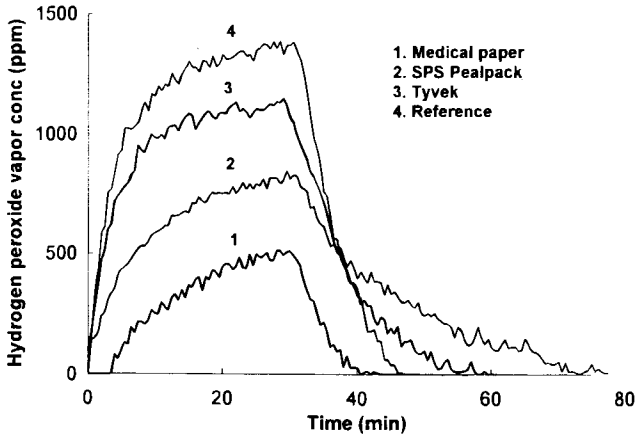

Fig. 4. Concentration-time profiles of hydrogen peroxide vapor (ppm) inside the isolator and in three different sterilization bags during VHP sterilization: medical paper (1), SPS Pealpack® (2), Tyvek® (3) and inside the isolator (4).

CONCLUSIONS

Near-infrared (NIR) spectroscopy using a gas cell connected with optic fibres is a useful technique to monitor VHP sterilisation cycles. By dectecting the absorbance at 1364, 1378 and 1400 nm the actual water vapor concentration can be measured while the actual hydrogen peroxide vapor can be detected by measuring the absorbance at 1420 nm. This approach is a useful technique in sterilisation cycle optimisation, validation and routine monitoring of VHP sterilisation cycles. The use of

NIR in evaluating the aeration time limits in range the low hydrogen peroxide vapor concentration. There was a difference in H_2O_2 vapor permeability of different packaging materials, commonly used in steam and ethylene oxide sterilisation.

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