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Locating bomb factories by detecting hydrogen peroxide

Francesco Saverio ROMOLO^{1,2}, Samantha Connell¹, Carlotta Ferrari¹, Guillaume Suarez³, Jean-Jacques Sauvain³, Nancy B. Hopf³

¹Institut de Police Scientifique (IPS), Université de Lausanne, 1004 Dorigny – Lausanne, Switzerland.

²Legal Medicine Section, Department SAIMLAL, SAPIENZA Università di Roma, Viale Regina Elena, 336, Rome, Italy.

³Institute for Work and Health (IST), 1066 Epalinges – Lausanne, Switzerland.

Abstract

The analytical capability to detect hydrogen peroxide vapour can play a key role in localizing a site where a H₂O₂ based Improvised Explosive (IE) is manufactured. In security activities, it is very important to obtain information in a short time. For this reason, an analytical method to be used in security activity needs portable devices. The authors have developed the first analytical method based on a portable luminometer, specifically designed and validated to locate IE manufacturing sites using quantitative on-site vapour analysis for H₂O₂. The method was tested both indoor and outdoor. The results demonstrate that the detection of H₂O₂ vapours could allow police forces to locate the site, while terrorists are preparing an attack. The collected data are also very important to develop new sensors; able to give an early alarm if located at a proper distance from a site where an H₂O₂ based IE is prepared.

Keywords: Precursor, Improvised Explosive (IE), Improvised Explosive Devices (IED), Home Made Explosive (HME), security, hydrogen peroxide, luminol,

Introduction

The study of precursors used to produce explosives, is a forensic field of increasing importance. These precursors allowed home-made preparations of improvised explosives (IE) used in several recent bombings [1-4], possibly including the recent terrorist attacks in Paris [5]. The European Parliament and the Council adopted the Regulation (EU) No. 98/2013 on the marketing and use of explosives precursors on 15 January 2014 [6]. According to this regulation, seven precursors shall not be available to the general public anymore in

concentrations greater than their limit values listed in Table 1. Despite the importance of the subject, few articles can be found in the scientific literature when searching with the keywords “explosive” and “precursor”.

	ANNEX I Regulated Substance	Limit Value (w/w)
1	Hydrogen Peroxide	12%
2	Nitromethane	30%
3	Nitric acid	3%
4	Potassium Chlorate	40%
5	Potassium Perchlorate	40%
6	Sodium Chlorate	40%
7	Sodium Perchlorate	40%

Table 1. Substances available to the public at concentrations equal or lower than the limit value according to Regulation (EU) No. 98/2013 of the European Parliament and of the Council on the marketing and use of explosives precursors (Annex 1) [6].

Chung et al. developed a methodology to prioritise potential precursor chemicals in order to assess the urgency of controls based on commodity chemicals being controlled under Korean regulations [7]. Considering analytical papers, standoff deep Raman allowed remote detection of concealed explosive precursors such as nitromethane and ammonium nitrate [8]. Ali et al. detected ammonium nitrate, hexamethylenetetraamine and pentaerythritol on clothing by Raman microscopy, after spotting particles with optical microscopy [9]. Nazarian and Presser used a laser-driven thermal reactor to obtain the thermal/chemical signatures of nitromethane and ammonium nitrate [10]. Finally Lazarowski and Dorman studied the capability of trained detection dogs to correctly signal the presence of one or more explosive mixtures containing potassium chlorate [11]. In addition, some authors have evaluated an isotopic analysis application to study a possible association between precursors used as starting material and explosive products obtained [12-15].

The scientific literature search was later focused on hydrogen peroxide (H_2O_2). This explosive precursor is of particular importance after its involvement in the foiled plot to blow up several aircrafts during their flights from London-Heathrow Airport. In this case, H_2O_2 was used to prepare homemade liquid explosives [16]. This kind of explosive, capable of detonating [17], was extensively studied in UK during the investigation and the criminal trial [18, 19]. Hydrogen peroxide (aq) has been detected through containers or packaging using Raman spectroscopy

[20, 21]. Ramirez-Cedeno et al. reported analyses of H_2O_2 concealed in mixtures with alcoholic beverages, such as whiskey, rum, and tequila via Raman [22]. Petterson et al. demonstrated the standoff detection capability of Raman with 5% H_2O_2 solutions in water through coloured glasses and PET at 30 m [23]. Stewart et al. proposed a handheld Raman spectrometer to determine H_2O_2 concentrations in liquids found at suspected IE manufacturing sites [24].

An online analyser was developed and used by Francois et al. in the field to measure atmospheric H_2O_2 obtaining values down to $3.5 \times 10^{-1} \mu\text{g m}^{-3}$ (177 pptv) [25]. Cotton swabs used for sample collection of hydrogen peroxide post-blast have been analysed using reversed-phase high-performance liquid chromatography with either fluorescence detection (HPLC–FD) or electrochemical detection (HPLC–ECD) [26, 27].

With the new H_2O_2 limit concentration for the products available to the general population (12%), criminals intending to use H_2O_2 to produce IE are forced to carry out a concentration process, as was done in 2005 in a flat in New Southgate, London. Terrorists allegedly used 443 litres of a commercial product containing hydrogen peroxide (18% concentration), and to have concentrated the H_2O_2 in heated pans in the kitchen to prepare the IE. Heating such liquids is expected to disperse significant H_2O_2 vapours into the surrounding air continuously throughout the production phase of the IE [28]. The detection of such vapours could allow police forces to locate sites, where the on-going manufacturing of a H_2O_2 based IE is suspected. This approach for protecting citizens from bombings is expected to be more effective than simply patrolling a possible target, because the production time of IE is much longer than the time needed to transport an improvised explosive device (IED) close to the target from the manufacturing site [29]. To our knowledge, no method has been specifically designed and validated to locate IE manufacturing sites using quantitative on-site vapour analysis for H_2O_2 . The aims of the research described in this paper were to develop a sampling and on-site detection method allowing low levels of H_2O_2 vapour to be measured for security reasons. This method was further tested at a military test site, where H_2O_2 vapours were emitted from a bomb factory kitchen.

Materials and Methods

Safety note

A literature and media review was completed to become familiar with the typical procedures used to concentrate commercially available H_2O_2 , which is a dangerous material. The handling of this substance in general and the activity described in this article in particular may only be carried out by authorised and highly qualified personnel, using appropriate safety measures (reinforced goggles and gloves, splinter-proof vessels, protective shield, etc.). Moreover, in Italy “It is forbidden to manufacture, to hold at home or elsewhere, to transport or to sell ... explosives that have not been recognised and classified by the Minister of the Interior” [30], such as mixture containing H_2O_2 .

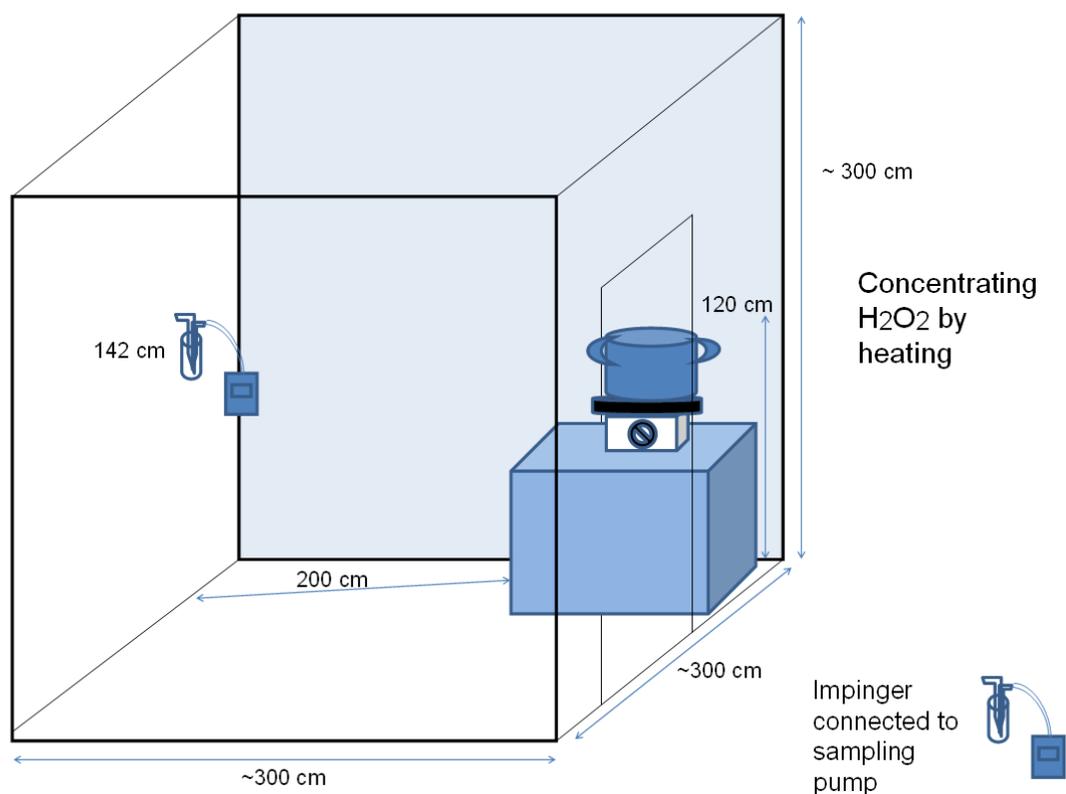
Materials

Hydrogen peroxide (12%) was purchased from a general store and H_2O_2 (30%) from Sigma Aldrich (5L, ref. 16911-5L-F, St. Louis, MO, USA). A 12 L stainless steel casserole (24 cm diameter) was also bought from a general store. A hot plate with a magnetic stirrer (Bibby, HC1202), a glass laboratory thermometer, and an oscillating fan operating statically (Sanyo) were used. A weather station was used to determine wind direction in field experiments (IROX model PRO-X2, ref. #IR4.1769.30).

Approach

To estimate H_2O_2 vapour emissions during concentration steps via heating, two approaches were used; a controlled laboratory experiment and a mock test. Three tests were performed in a controlled environment [31] at the Institute for Work and Health (IST) (Lausanne, Switzerland). Hydrogen peroxide sampling at different distances from the source were carried out inside a closed and ventilated exposure chamber (10 m^3) (Figure 1). The capability of the developed method to detect H_2O_2 vapours in field conditions was tested at the Armasuisse, Science & Technology Competence Center in Thun, Switzerland, where an IE manufacturing site was created and used as a model. The drawing in Figure S1 of the supplementary material section shows the experimental set-up on-site with the positions of the IE manufacturing site and the sampling units located along the direction of the wind.

Figure 1. Drawing depicting exposure chamber experimental set-up at IST.



Air sampling

Air samples were collected using a personal air sampling pump (SKC AirChek 500 or SKC AirChek 224 PCXR4; Eighty Four, PA, USA), connector tubing (Tygon[®]), and glass impingers (SKC Fritted Midget Impinger, Glass, 25 mL, with Standard Nozzle) containing water (15 mL from Milli-Q Q-POD, Switzerland, 18.2 M Ω .cm, < 4 ng/g total organic carbon, filtrated through 0.22 μ m filter) operated at a flow rate of 1 litre per minute (Lpm) (Figure 2).

Figure 2. Photo of sampling train used to sample H₂O₂, attached to poles at increasing distances, as shown in Figure S1 of the supplementary material section.



Chemiluminescent Method Development

Bench-top analysis

Chemiluminescence methods were sensitive to H_2O_2 and appeared convenient for the envisioned application. The method described here corresponds to the combination of two published chemiluminescent (CL) methods for H_2O_2 [32], [33], and one for peroxide-based

explosives [34]. A horseradish peroxidase (HRP) and luminol mixture was used to react with H_2O_2 and produce a light emission, which was analysed by a luminometer, providing quantitative results. Preliminary tests to determine sensitivity of two types of HRP in buffer solutions (VI in PBS and X in Tris) were carried out.

- HRP type VI (from horseradish, Sigma Aldrich, Switzerland, N° P8375-1KU) in Phosphate buffer (PBS), pH 7.4 (Bichsel AG, Interlaken Switzerland) or in 0.1 M Tris buffer (pH 8.4)
- HRP type X (from horseradish, ammonium sulphate suspension, Sigma Aldrich, Switzerland, N° P6140-2KU) in 0.1 M Tris buffer (pH 8.4)

The optimised reaction mixture for the bench-top luminometer consisted of mixing luminol (5 mL, 10 mM in 0.1 M Tris buffer pH 8.4) and HRP type X (10 mL, 5 U/mL in 0.1M Tris buffer pH 8.4). A microplate reader in CL mode (Tecan Infinite M200, Männedorf, Switzerland) was used to quantify the H_2O_2 concentrations. The microplate reader results were considered the reference value. Samples (standard H_2O_2 solution (250 μ L, 0-3 μ M) or sample (250 μ L)) were introduced in defined wells in a white 96 well microplates (Nunclon Delta N° 136101).

The optimised HRP/luminol mixture (10 μ L) was then injected in the well, followed by measurement of the luminescence intensity every 20 seconds during a total time of 15 minutes (45 cycles). The integration of the emitted light over time was related to the H_2O_2 concentration in the sample. The calibration curve fitted a second order polynomial function forced to zero, consistent with literature data [32].

On-site analysis by portable luminometer

For on-site measurement, H_2O_2 concentrations were measured using a portable luminometer (Hygienia EnSURE, Camarillo, CA, USA). As this instrument is fully manual, it is not possible to record the light emission immediately after addition of the HRP/luminol mix. Some delay (allowing for shaking and introducing the sample in the instrument) was needed before measurement started. Preliminary tests were performed to optimise the light emission by modifying the HRP/luminol ratio. Four HRP/luminol mix (ratio 5/2.5; 2.5/5; 1.25/6.25; 0.625/6.375 volume:volume) of HRP Type X 5 U/mL and luminol 10 mM in Tris buffer (pH 8.4) were prepared and tested and the final optimised concentrations of the HRP/luminol mixture used with the portable luminometer corresponded to mixing 1.25 mL HRP type X 5U/mL in 0.1M Tris buffer pH 8.4 with 6.25 mL luminol 10 mM in 0.1M Tris buffer pH 8.4.

Original Aquasnap tubes (Aquasnap[®] ATP water test, Art. AQ-100X) were modified by cutting the mixing wand to optimize their use in this project. To avoid possible contamination, the tubes were washed with deionized water (three times per tube) and methanol (one time per tube), followed by air drying before use. Hydrogen peroxide standard solutions (0-4 μM) or impinger samples (500 μL) were added to a pre-washed tube. The optimised HRP/luminol mix (30 μL) was added to the sample, the tube shaken manually to ensure well mixing, inserted into the portable luminometer and analysed. Five seconds elapsed from adding the luminol mix to the start of the readings. After 15 seconds, the amount of light emitted (in arbitrary units) was recorded, followed by seven additional light measurements over the next 120 seconds, manually read on the portable luminometer, shown in Figure S2 of the supplementary material section. The sum of these eight measurements corresponded to the total light emitted during two minutes. Each measurement was performed in triplicate. Tubes were washed as above and reused after each experiment.

Experimental Set-up

In the experiments performed in the exposure chamber, an initial volume of H_2O_2 (aq) was measured in a glass cylinder and poured into a pot on top of an electric hot plate. The initial volume of aqueous H_2O_2 to concentrate, varied by experiment, ranging from 2.1 to 3.6 L. The initial H_2O_2 (aq) concentration varied by experiment, ranging from 4.5 to 7 M. The heating procedure lasted a minimum of 4 hours and final H_2O_2 concentrations could be as high as 24.5 M in the pot. Air samples were collected every 20 minutes, then capped, and stored under refrigeration until analysis. Curves of increasing H_2O_2 concentrations in air over time were generated.

The field test simulating H_2O_2 concentration in order to understand the environmental concentration profile and the effect of dilution was carried out at Armasuisse, Science & Technology Competence Center in Thun, Switzerland. Four litres of 30% H_2O_2 was concentrated for approximately 4.5 hours with the same procedure adopted for the laboratory experiments performed in the exposure chamber. The source was located approximately 0.5 m from the bomb factory window. A total number of 20 air samples were collected. Sampling trains were taped to metal poles at 155 cm height and placed at distances 0.5 – 20 meters from the source in the direction of the wind (Figure 3). Sample times and distances of the

impingers from the source were based on real-time results obtained by the portable luminometer. As positive results were received at close distances with the direct-reading instrument, the stationary samplings were moved further away from the source, and sampling times were increased with distance. Sampling times ranged from 15 – 50 minutes.

Figure 3. Photo of sampling poles located at increasing distances from the source.



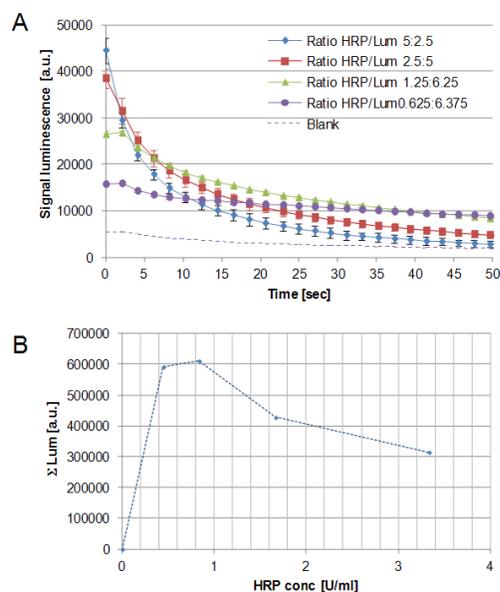
Results and discussion

Preliminary tests regarding the effect of the HRP type on the chemiluminescence signal were measured using H_2O_2 standard solutions. Due to solubility problems for luminol at pH 7.4, tests were performed only at pH 8.4 with 0.1M Tris buffer. Whereas luminescence intensities were always slightly higher for HRP type VI compared to HRP type X, no statistically significant luminescence differences for the H_2O_2 concentration above $1 \mu\text{M}$ were observed between the two HRP mixtures tested (Student t-test, $p > 0.05$). Figure S3 in the supplementary material section shows the calibration curves for H_2O_2 standard solution with HRP/luminol mixture either with HRP Type VI (blue curve) or HRP Type X (red curve). Therefore HRP type X was used for all other following chemiluminescence measurements.

The portable luminometer doesn't allow the injection of the reactive HRP/luminol mix directly

inside the measurement cell. Mixing the sample with the HRP/luminol mix must therefore be done before any measurement. This delay means that some of the emitted light was lost. This loss should remain as low as possible in order to be sensitive enough to measure trace levels of H_2O_2 . By changing the ratio of the enzyme to luminol, the light emission could be modulated as illustrated in Figure 4. By decreasing the HRP concentration in the mixture, the initial light emission signal clearly decreased but was more constant over time, as illustrated in Figure 4A. The integration values of the emitted light during the first 150 seconds of reaction as a function of the HRP concentration in the reacting media is shown in Figure 4B. The total light emitted was the greatest for a mixture containing about 0.8 U/mL HRP Type X and 8.3 mM luminol in 0.1 M Tris buffer pH 8.4. These conditions (corresponding to 1.25 mL HRP 5U/mL + 6.25 mL luminol 10 mM) were adopted for all the measurements taken with the portable luminometer.

Figure 4 - Testing the ratio of HRP Type X/luminol (volume:volume) on the luminescence kinetic. A): luminescence decay for the different volume ratio; B): Integration of the emitted light during the first 150 seconds of reaction as a function of the HRP concentration in the reacting media. Measurements were done with a standard solution of H_2O_2 0.97 μM .

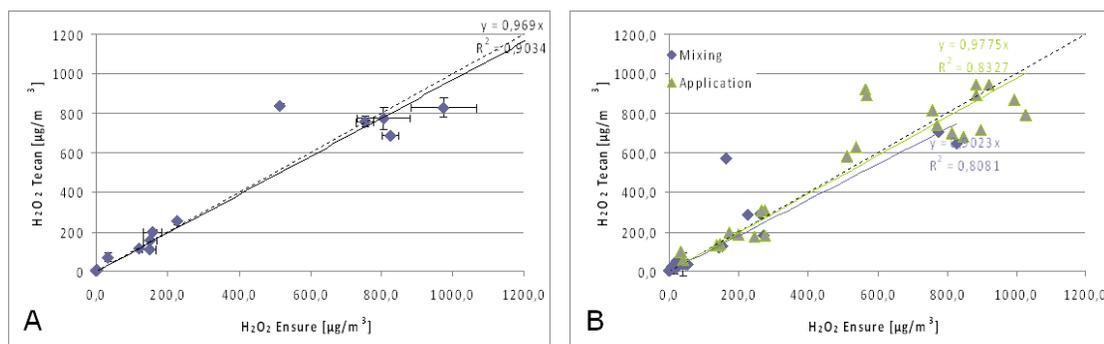


The limit of detection (LOD), corresponding to three times the standard deviation of the signal for a H_2O_2 0.1-0.4 μM standard solution, was 0.1 ± 0.03 μM with the bench-top luminometer and it was 0.2 ± 0.03 μM for the portable luminometer. The LOD for the portable luminometer was also evaluated later, considering a coefficient of variation larger than 30% for duplicate measurement at the LOD. With this approach, a LOD of 0.25 μM was obtained.

When expressed as air concentration, the LOD for the bench plate reader and the portable luminometer translated into 3.4 $\mu\text{g}/\text{m}^3$ and 6.8 $\mu\text{g}/\text{m}^3$ respectively. The precision of the portable luminometer method ($\text{CV} < 10\%$) was 1 μM H_2O_2 (aq). Calibration curves were obtained over four days, resulting in high repeatability. Figure S4 in the supplementary material section show the high repeatability in H_2O_2 calibration using the portable luminometer over four days.

The comparison between the luminescence methods (the microplate bench-top reader and the portable luminometer) gave a very good correlation, obtained by plotting the H_2O_2 concentrations in the impinger obtained with both methods (Figure 5). The concordance between the two measurements by using the Bland-Altman plot was good, with a mean of the difference of -6 $\mu\text{g}/\text{m}^3$ (95% confidence limit between +26 and -39 $\mu\text{g}/\text{m}^3$). Figure S5 in the supplementary material section shows the Bland-Altman plot demonstrating concordance between H_2O_2 levels using the bench-top instrument and portable luminometer.

Figure 5 - Relationship between the H_2O_2 results obtained with the bench-top instrument (Tecan) and the portable luminometer (EnSURE). Picture A corresponds to an « averaged » concentration over 15 minutes. Figure B corresponds to two steps: one in blue, averaged concentration over 5 minutes sampling ; and another in green, averaged concentration over 10 minutes sampling.

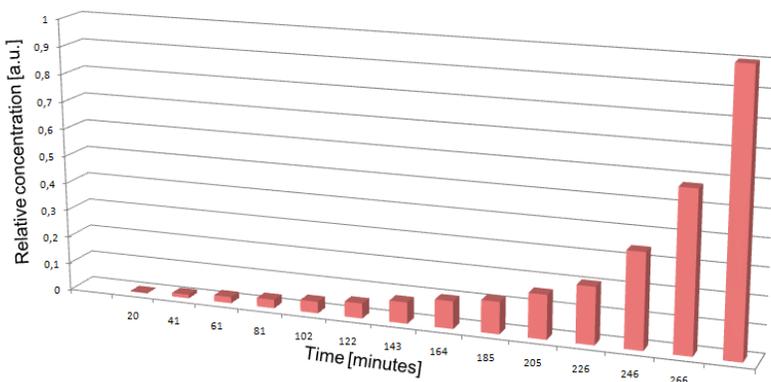


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Test Results

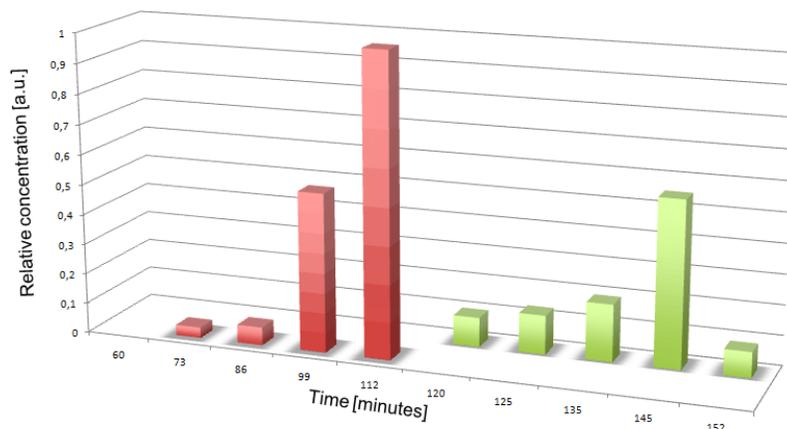
During the three exposure chamber experiments, the initial H₂O₂ aqueous solutions concentrated in the pot ranged from 5 to 12% and final concentrations ranged from 64 to 84% at the end of the experiments. Fifty-eight air samples were collected during these experiments. The sampling time was always 20 minutes. An example of the evolution of the H₂O₂ concentration (in arbitrary units) over time indoor is shown in Figure 6.

Figure 6 –H₂O₂ concentrations in air over time (x axis; in minutes) measured using an impinger. The concentration is reported in arbitrary units normalised to the highest concentration obtained in an indoor test (i.e. the maximum y value is 1 in arbitrary units).



The proposed method was further tested at a military test site, where H_2O_2 vapours were emitted from a bomb factory kitchen. Twenty air samples were collected during this test. In both cases the concentration data obtained are confidential EU. For this reason they are reported in Figure 6 and in Figure 7 in arbitrary units normalised to the highest concentration obtained. Despite the understandable limit of confidentiality, we describe here a whole method of making tests, of sampling and of obtaining on-site quantitative data, needed to develop new sensors able to give an early alarm if located at a proper distance from a site where an H_2O_2 based IE is being prepared.

Figure 7 – H_2O_2 concentrations over time (x axis; minutes) in two impingers, positioned at distance a (in red) and distance b (in green) from the source, with $b > a$. The concentration is reported in arbitrary units normalised to the highest concentration obtained in the outdoor test (i.e. the maximum y value is 1 in arbitrary units).



The concentration experiments demonstrated that when hydrogen peroxide is concentrated by heating, a significant amount of hydrogen peroxide in vapour phase is released from the pot into the surrounding air, both indoor and outdoor.. As expected, concentrations generated in the chamber were greater due to the confined (non-diluted) area compared to the field results. Air sampling is highly affected by environmental conditions, especially by the wind direction and speed, resulting in extensive dilution of the contaminant in the environment. Despite outdoor dilution, the results obtained showed that it is possible to detect H_2O_2 vapours using the proposed on-site analytical approach, allowing possible detection during the manufacturing of an H_2O_2 based IE. The detection of such vapours could allow police forces to locate the site, where terrorists are preparing an attack, during the long time needed to prepare the charge. The collected data can also be used to estimate H_2O_2 concentrations that could be found at some distance from the source, in the case of an artisanal bomb factory.

Conclusion

After the adoption of the European Parliament and the Council Regulation (EU) No. 98/2013 on the marketing and use of explosives precursors in January 2014, there is a new interest about on-site precursor detection for security purposes. We have focused the attention on hydrogen peroxide, which is a building block involved in several terrorist attacks in recent

history. Our developed approach targets the time the terrorists need to concentrate H_2O_2 when H_2O_2 vapours can be measured in air. We have developed for the first time an analytical method specifically designed to provide quantitative on-site analysis for airborne H_2O_2 . The method, based on a portable luminometer, was validated and tested both indoors and outdoors. In the latter case, H_2O_2 vapours were emitted from a bomb factory kitchen on a military base.

The analytical procedure proposed is not only a considerable tool to protect the security of citizens by terrorist attacks and to support the activities of Police Forces; but it is also very important to support the development of new sensors, aiming to locate Improvised Explosive (IE) manufacturing sites. an early alarm of the related threat.

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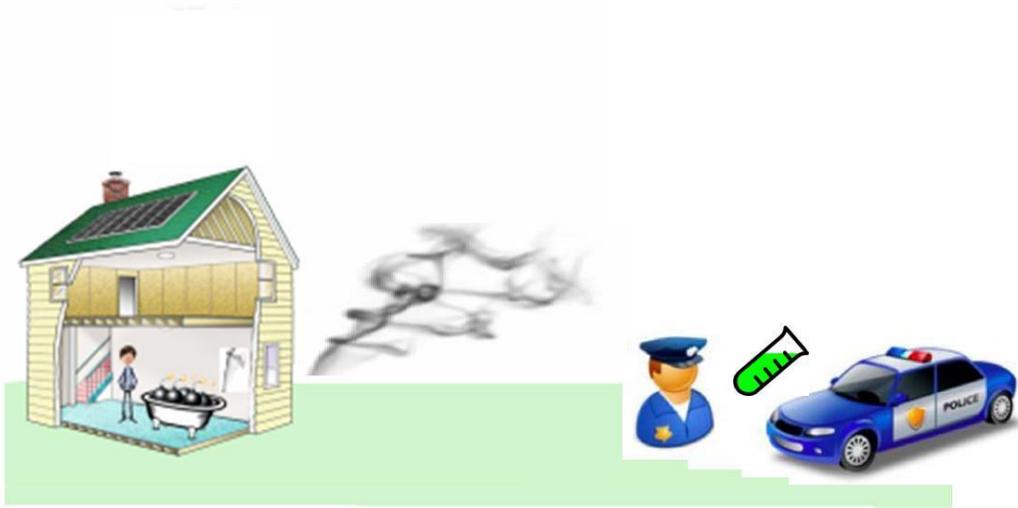
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Highlights

- A bomb factory can be located by analysing H₂O₂ vapours emitted.
- A suitable quantitative on-site vapour analysis for H₂O₂ is proposed.
- A portable luminometer can effectively support the activities of Police Forces.

Graphical abstract.



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