



Received on 11 November, 2012; received in revised form, 23 December, 2012; accepted, 15 February, 2013

DETECTION OF ETHYLENE FOR *IN VIRO* EVALUATION OF THE UV PHOTOPROTECTION

I. E. Santosa¹, A.N.I. Harnita², S. Martono³, Sudarsono³, S. Widayari⁴ and F.J.M. Harren*⁵

Department of Physics Education¹, Faculty of Pharmacy², Sanata Dharma University, Yogyakarta, Indonesia
Faculty of Pharmacy³, Faculty of Veterinary Science⁴, Gadjah Mada University, Yogyakarta, Indonesia
Life Science Trace Gas Facility, Molecular and Laser Physics, Radboud University⁵, Nijmegen, the Netherlands

Keywords:

Lipid peroxidation, ROS, UV, ethylene

Correspondence to Author:

F.J.M. Harren

Life Science Trace Gas Facility,
Molecular and Laser Physics, Radboud
University, Nijmegen, the Netherlands

E-mail: f.harren@science.ru.nl

ABSTRACT: In this study, the antioxidant capacity during UV exposure has been investigated by using a laser-based trace gas detector for the detection of gaseous ethylene as an indicator for lipid peroxidation. Biocream was used as a control and ascorbic acid, butylated hydroxytoluene, phloroglucinol and hydroquinone were added as antioxidants in a mixing concentration of 2.5% (w/w). The experimental results show that there is a decrease in the number of ROS due to the presence of antioxidants, from which ascorbic acid is the most effective. The detection method offers a direct and on-line way to evaluate the capability of antioxidant in reducing the harmful effect of the UV radiation.

INTRODUCTION: In the past various *in vitro* and *in vivo* methods have been presented to evaluate the photo protection efficacy of sunscreen based on the measurement of sun protection factor (SPF), UVA/UVB ratio, and their critical wavelength^{1, 2, 3, 4, 5}. The determination of SPF *in vivo* is time consuming and expensive; in addition, the method is more suitable for testing the effect of UVB radiation due to its exclusion of UVA radiation^{4,5}.

For testing the efficacy of sunscreen against UVA various *in vitro* and *in vivo* methods have been proposed, such as spectrophotometric based methods measuring the UVA absorption of sunscreens^{2, 6}. However, in the latter method the photochemical reaction within the sample as a result of UVA exposure is not considered.

Other research show that during the exposure of UVA on the skin, various reactive oxygen species (ROS) are generated; such as the presence of ROS using ESR (Electron Spin Resonance)^{7,8}. UVA radiation stimulates the generation of ROS, which itself induces lipid peroxidation in skin tissue.

By investigating the end products of lipid peroxidation the presence of ROS can also be justified. In the past lipid peroxidation was investigated using the well known TBARS (ThioBarbituric Acid Reactive Substances) method^{9,10}, measuring malondialdehyde (MDA) from homogenized samples to prove the occurrence of lipid peroxidation. It is known that the lipid peroxidation process produces various gases such as ethylene and ethane⁹. As gaseous product of lipid peroxidation during UV illumination on the skin ethylene has been investigated before, using a laser-based detection method^{11,12,13,14}. Monitoring the end product of lipid peroxidation via gas emissions provides an alternative and powerful method, due to its on-line and non invasive capabilities.

In this study, we will use the same laser-based photoacoustic detection method for the evaluation of the protection ability of antioxidant for UV radiation. This protection capability of antioxidant is tested by measuring gaseous ethylene released during UV irradiation as an indicator for the lipid peroxidation process.

MATERIALS AND METHODS:

Materials: In this study Biocream (Merck, Germany) was used as a blank media. This Biocream is an amphiphilic dermatological cream and contains aerosil, parafin. liq., vaselin. album, cetostearol., sorbimacrogol. palmit. 300, monostearin., Miglycol 812, acid. sorbic., propylen-glycol., and deionized water. In this Biocream ascorbic acid, butylated hydroxytoluene (BHT), phloroglucinol and hydroquinone were mixed as antioxidant. These chemicals were of highest grade commercially available from Merck Germany, and were mixed into the Biocream in a 2.5% (w/w) concentration.

Instrumentation: The ethylene production from the Biocream under exposure of UV radiation was measured using a commercial ethylene detector (Sensor Sense BV, Nijmegen, the Netherlands). As UV radiation source a Philips original home solaria HB 405/A was used containing a Philips HPA 400W UV type 3 lamp. The UV intensity was measured with Dr Honle AG-a UV meter. A specially designed aluminum cuvette¹⁵ was used for this experiment with a large surface/volume ratio (diameter 4.6 cm, height 0.6 cm) as shown in **Fig. 1**.

On top the cuvette was covered with a quartz window permitting UV radiation reaching the Biocream mixture. A rubber o-ring was used to between the window and the aluminum to seal the inner volume

from outside air. Inlet and outlet ports allow a carrier gas to flow over the cream during UV exposure and transport the emitted ethylene to the laser-based gas detector for on-line detection. In addition the temperature of the cuvette was maintained constant, using a temperature controlled water bath

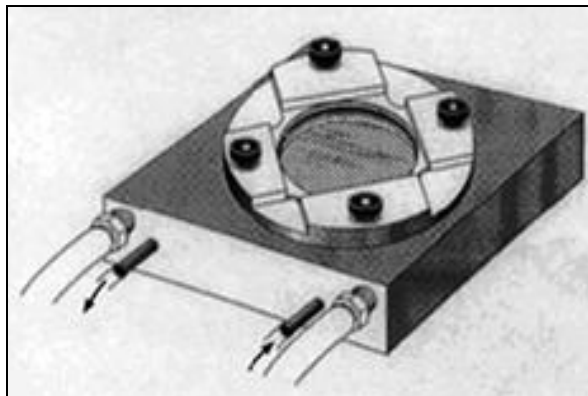


FIGURE 1: The sample cuvette consists of an aluminum block with a chamber milled out of the upper side. A glass plate is placed at the bottom of the chamber with on top the sample. A quartz window on top of the chamber allows illumination of the sample. Arrows indicate the inlet and outlet ports for the carrier gas. The other tubes are water inlet and outlet for controlling the temperature of the chamber.

Procedure: The Biocream was smeared on a glass plate (4 cm diameter) over the area of 12.5 cm² and put into the cuvette. **Fig. 2** shows the overall experimental setup.

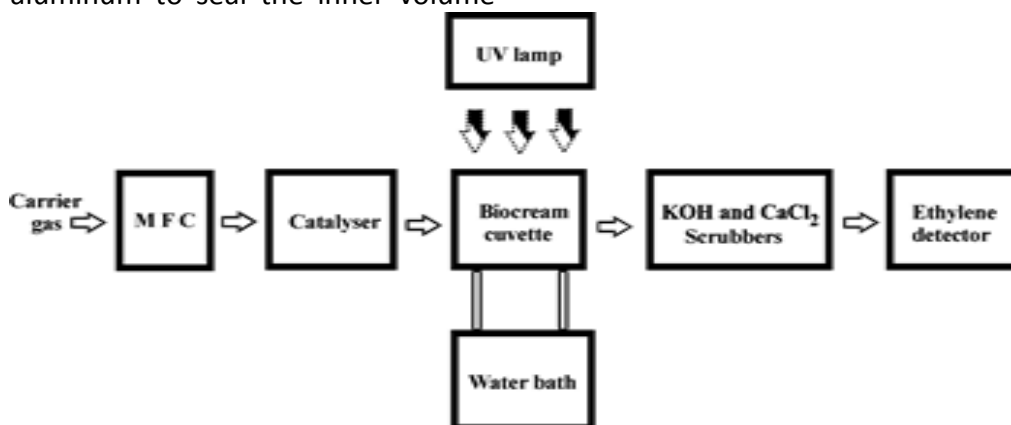


FIGURE 2: EXPERIMENTAL SETUP

A mass flow controller (MFC) controlled the carrier gas flow rate (in our case dry air) over the sample and was kept constant at 1.8 liter/hour. The incoming air contains low concentrations of ethylene (1-10 ppbv, part per billion volume), to avoid interference with generated ethylene during our experiments this was

removed from the airflow by a catalyst before entering the sampling cell together with other gaseous hydrocarbons^{16, 17}. CO₂ and water vapor can spectroscopically interfere with the infrared absorption spectrum of ethylene at the laser lines of the laser-based ethylene detector; therefore they were removed

from the airflow by KOH and CaCl_2 pellets, respectively. More accurate and sensitive measurements could be performed by using these scrubbers^{11, 17}. Using the ethylene detector, the ethylene concentration in the outgoing gas flow was measured continuously every 5 seconds. For each experiment the ethylene measurements started 5 minutes prior to the UV illumination to obtain an ethylene baseline. The measurements continued during UV illumination (10 minutes) and till 10 minutes after exposure, to allow completely refreshment of the ventilation volume.

RESULTS: Ethylene release from the Biocream was monitored continuously using the ethylene detector. In **Fig. 3**, a typical ethylene emission (nl/hour) pattern is shown under the influence of UV radiation. In this figure the black and white bar indicate when the UV lamp is turned off and on. The UVA and UVB irradiance at the position of the Biocream are (14.3 ± 0.4) mW/cm^2 and (1.6 ± 0.1) mW/cm^2 , respectively. A fast increase in ethylene emission (in nl/h) is detected two minutes after illumination. After leveling off the emission shows a stable value. The start of the decrease in ethylene emission is also observed two minutes after turning off the UV lamp.

The ethylene emission for various amounts of Biocreams (8.2 mg, 33.1 mg and 45.8 mg) under UV illumination is shown in **Fig. 4**. Since the surface area over which the Biocream is smeared stays the same the figure shows the effect of the penetration depth of the UV radiation into the Biocream and the effect of ethylene diffusion out of the cream. The thickness of the Biocream can be calculated from the area, mass, and the density of Biocream, i.e. $0.871 \text{ g}/\text{ml}$. In this experiment the thickness is between $9 \mu\text{m}$ and $54 \mu\text{m}$ for 10 mg and 60 mg of Biocream, respectively.

Despite the diffusion effects a linear relation can be found (**Fig. 5**) between the ethylene production and the Biocream mass resulting in a production rate measured in nl/g . Furthermore, we observed a linear dependence of ethylene emission with the UVA and UVB intensity, by changing the distance between the lamp and the cuvette (data not shown), which indicates that there is no bleaching or saturation effects occur in the medium.

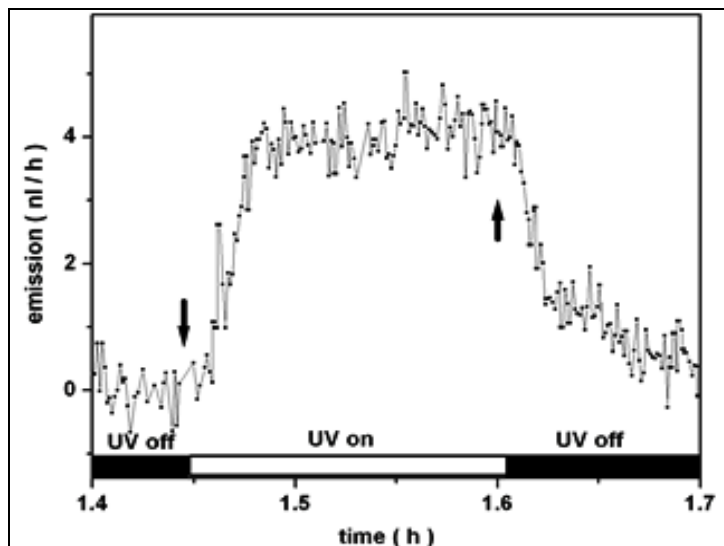


FIGURE 3: ETHYLENE EMISSION DURING UV ILLUMINATION PERIOD ON BIOCREAM

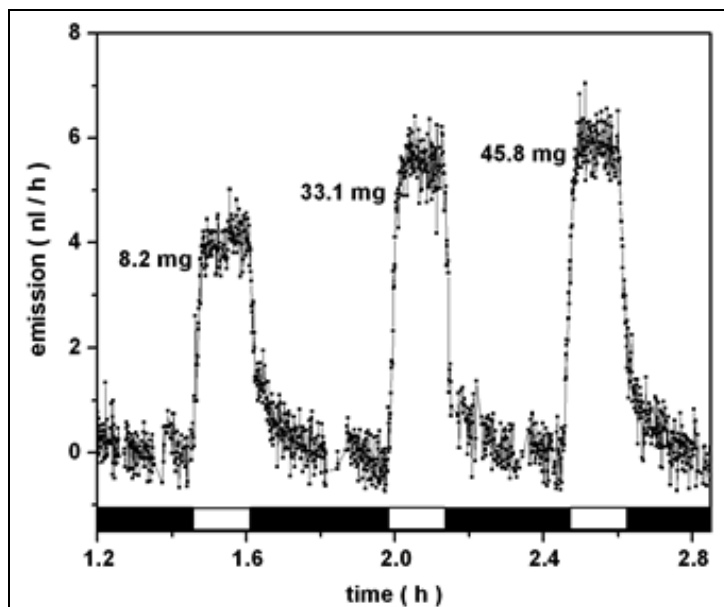


FIGURE 4: EMISSION OF ETHYLENE FROM DIFFERENT AMOUNTS OF BIOCREAM

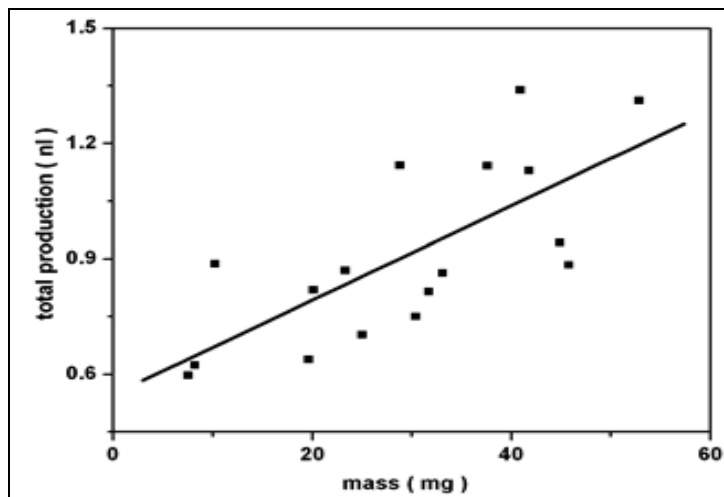


FIGURE 5: TOTAL PRODUCTION OF ETHYLENE FOR VARIOUS AMOUNTS OF BIOCREAMS

Various Biocrems mixtures with antioxidants were tested and their production rates are shown in **Table 1**. Table 1 shows that the highest production of ethylene comes from pure Biocream. If the Biocream contains an antioxidant it produces less ethylene, down to 25% of its original value. This indicates that antioxidants such as ascorbic acid, BHT, phloroglucinol and hydroquinone scavenge the ROS that has already been generated during UV irradiation. This effect resulted in lower concentrations of ROS and subsequently less lipid peroxidation. Ascorbic acid shows the most potent antioxidant activity.

TABLE 1: TOTAL ETHYLENE PRODUCTION FROM DIFFERENT SAMPLES

Sample	Ethylene production
Biocream	12.3 ± 3.0
Biocream, 2.5 % ascorbic acid	2.5 ± 0.8
Biocream, 2.5 % BHT	4.0 ± 1.0
Biocream, 2.5 % phloroglucinol	3.5 ± 0.5
Biocream, 2.5 % hydroquinone	5.2 ± 2.5

DISCUSSION: Fig. 3 shows that about two minutes after UV illumination the Biocream emits ethylene. Subsequently also two minutes after the UV lamp is turned off, this ethylene emission diminishes to zero. This two minute delay is mainly due to the gas transport time from the cuvette to the ethylene detector. The Biocream used in this experiment contains different compounds such as paraffin and Vaseline; therefore the sample is rich of lipids. During the UV illumination period (UVA: 14.3 ± 0.4 mW/cm² and UVB: 1.6 ± 0.1 mW/cm²) ethylene emission was detected from the sample and thus demonstrated that lipid peroxidation occurs.

Corresponding to this, it can be concluded that reactive oxygen species (ROS) are generated by UV radiation within the samples which is in agreement with previous reported results from ESR spectroscopy which detected UVA induced free radicals formation in the skin^{7,8}. The total ethylene production depends on the amount of lipids (mass of the Biocream) that has been affected by ROS (Fig. 5). From this figure it can be seen that the individual experiments show some spread, which is most probably due to the non-homogeneity of the sample thickness on the glass plate. In the experiment the mass of the samples can be measured accurately, but not the variation of the thickness.

The figure shows a linear relation between the total production and the amount of lipids resulting in a production rate in nl/g.

Various Biocrems mixtures with antioxidants were tested and their production rates are shown in Table 1. It is shown that the antioxidants ascorbic acid, BHT, phloroglucinol and hydroquinone scavenge the reactive oxygen species resulting in less lipid peroxidation. Ascorbic acid shows the most potent antioxidant activity. These observations facts are in accordance with previous reported antioxidant activity of ascorbic acid, BHT, phloroglucinol and hydroquinone. BHT and ascorbic acid have been previously used as positive control for the free radical-scavenging activities using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) test. Ascorbic acid exhibited a higher activity than BHT in this test¹⁸. The radical scavenging activity of DPPH can be expressed in an oxidation index EC₅₀ (the effective concentration to scavenge 50%)¹⁹. For ascorbic acid and BHT these are 0.09 mg/ml and 0.16 mg/ml, respectively. These EC₅₀ are smaller than for α -tocopherol. Zubia *et al.*,¹⁹ also found that superoxide anion radical scavenging activity of ascorbic acid is higher than BHT.

Shibata *et al.*,²⁰ evaluated the antioxidant activities of various compounds using the inhibition of phospholipid peroxidation in the liposome system, and by determining their radical scavenging activities against the superoxide anion and DPPH. They found that the ascorbic acid is more effective than phloroglucinol in radical scavenging activities against the superoxide anion and DPPH. Furthermore ascorbic acid and phloroglucinol show about 20% and 10% inhibition of phospholipid peroxidation²⁰.

Also the antioxidant activity of hydroquinone has been investigated using DPPH test. Hydroquinone showed more potent DPPH radical-scavenging activity than arbutin (a herbal medicine) and their EC₅₀ concentrations were 0.02 and 0.05 mM, respectively²¹. Fujisawa and Kadoma²² investigated the oxidation of α -tocopherol and hydroquinone using differential scanning calorimetry. Both compounds trapped approximately two radicals per molecule and the rate of inhibition of polymerization (chain propagation) was greater for hydroquinone than for α -tocopherol.

In contrary to these previous investigations, this study detects one of the end products of lipid peroxidation and is therefore a direct method to show the damaging effect of UV irradiation. One of the end products is ethylene which is used in our experiment as marker of the quality of antioxidant as protective compound against UV radiation.

CONCLUSION: In this investigation we have detected ethylene as the end product of lipid peroxidation. The ethylene detection has been used for indicating the presence of UV stimulated ROS in Biocream. The experimental results show that there is a decrease in the number of ROS due to the presence of antioxidants, from which ascorbic acid is the most effective. The detection method offers a direct and on-line way to evaluate of the capability of antioxidant in reducing the harmful effect of the UV radiation.

ACKNOWLEDGMENTS: The authors thank to Sacco te Lintel Hekkert for providing the ethylene detector and Cor Sikkens for his expert technical assistance. Financial support by the KNAW (The Royal Dutch Academy of Science and Art) is gratefully acknowledged.

REFERENCES:

- Schalka, S., Reis V. M. S., Cuce L. C. The influence of the amount of sunscreen applied and its sun protection factor (SPF): evaluation of two sunscreens including the same ingredients at different concentrations. *Photodermatol Photoimmunol Photomed.* 2009; 25:175-180.
- Diffey, B. Spectral uniformity: a new index of broad spectrum (UVA) protection. *Int J Cosmet Sci.* 2009; 31:63-68.
- Velasco, M. V. R., Sarruf F. D., Santos I. M. N. S., Filho C. A. H., Kaneko T. M., Baby A. R. Broad spectrum bioactive sunscreens. *International Journal of Pharmaceutics.* 2008; 363:50-57.
- Bleasel, M. D., Aldous S. In vitro evaluation of sun protection factors of sunscreen agents using a novel UV spectrophotometric technique. *Int J Cosmet Sci.* 2008; 30:259-270.
- Petrazzuoli, M. Advances in sunscreens. *Curr Probl Dermatol.* 2000; 287-290.
- Cole, C. Sunscreen protection in the ultraviolet A region: how to measure the effectiveness. *Photodermatol Photoimmunol Photomed.* 2001; 17: 2-10.
- Herrling, T., Fuchs J., Rehberg J., Groth N. UV-induced free radicals in the skin detected by ESR spectroscopy and imaging using Nitroxides. *Free Radic Biol Med.* 2003; 35:59-67.
- Herrling, T., Jung K., Fuchs J. Measurements of UV-generated free radicals/reactive oxygen species (ROS) in skin. *Spectrochim Acta A.* 2006; 63: 840-845.
- Halliwell, B. and Gutteridge J. M. C. *Free Radicals in Biology and Medicine.* Oxford University Press, Inc, New York. 1999.
- He, Y. Y., Klisch M., Hader D. P. Adaptation of cyanobacteria to UV-B stress correlated with oxidative stress and oxidative damage. *Photochem Photobiol.* 2002; 76(2):188-196.
- Harren, F. J. M., Berkelmans R., Kuiper K., te Lintel Hekkert S., Scheepers P., Dekhuijzen R., Hollander P., Parker D. H. On-line laser photoacoustic detection of ethene in exhaled air as biomarker of ultraviolet radiation damage of the human skin. *Appl Phys Lett.* 1999; 74(12): 1761-1763.
- Cristescu, S., Berkelmans R., te Lintel Hekkert S., Timmerman B., Parker D., Harren F. Photoacoustic trace gas detection of ethene released by UV-induced lipid peroxidation in humans. *Proc of SPIE* 4162. 2000;101-105.
- Timmerman, B. H., Berkelmans R., Cristescu S., te Lintel Hekkert S., de Gruijl F., Meijers M., Harren F. J. M., Gerritsen R., Parker D. In-vivo studies of UV effects in human skin using photoacoustics. *European UV sunfilters Conference Proc.* 1999;19-25.
- te Lintel Hekkert, S., Berkelmans R., Kuijper K., Scheepers P., Hollander P., Harren F. J. M., Parker D. H. On-line, laser based, detection of ethene in exhaled air as indicator for UV induced skin damage. *European UV sunfilters Conference Proc.* 1998; 36-39.
- Staal, M., te Lintel Hekkert S., Harren F., Stal L. Nitrogenase activity in cyanobacteria measured by the acetylene reduction assay: a comparison between batch incubation and on-line monitoring. *Environ Microbiol.* 2001; 3(5): 343-351.
- Eastwell, K. C., Bassi P. K., Spencer M. E. Comparison and evaluation of methods for the removal of ethylene and other hydrocarbons from air for biological studies. *Plant Physiol.* 1978; 62: 723-726.
- Bijnen, F. G. C., Zuckermann H., Harren F. J. M., Reuss J. Multicomponent trace-gas analysis by three intracavity photoacoustic cells in a CO laser: observation of anaerobic and postanaerobic emission of acetaldehyde and ethanol in cherry tomatoes. *Appl Opt.* 1998; 37(15): 3345-3353.
- Duan, X. J., Zhang W. W., Li X. M., Wang B. G. Evaluation of antioxidant property of extract and fractions obtained from a red alga *Polysiphonia urceolata*. *Food Chemistry.* 2006; 95: 37-43.
- Zubia, M., Robledo D., Pelegrin Y. F. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *J Appl Phycol.* 2007; 19: 449-458.
- Shibata, T., Ishimaru K., Kawaguchi S., Yoshikawa H., Hama Y. Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J Appl Phycol.* 2008; 20: 705-711.
- Bang, S. H., Han S. J., Kim D. H. Hydrolysis of arbutin to hydroquinone by human skin bacteria and its effect on antioxidant activity. *J Cosmet Dermatol.* 2008; 7: 189-193.
- Fujisawa, S., Kadoma Y. Differential scanning calorimetry investigation of the radical-scavenging activity of α -Tocopherol and Hydroquinone. *Instrumentation Science and Technology.* 2005; 33: 261-269.

How to cite this article:

Santosa IE, Harnita ANI, Martono S, Sudarsono, Widyarini Sand Harren FJM: Detection of Ethylene for *In vitro* evaluation of the UV Photoprotection. *Int J Pharm Sci Res.* 2013; 4(3); 983-987.