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Abstract. While much is known about the effect of smoke and vapors on the composition of blood, little is known about their impact on the composition of breath. When tobacco from traditional cigarettes (T) is burned, it produces harmful smoke compared with the vapor produced when using electronic cigarettes (E). Using a non-invasive, safe, and rapid CO₂ laser-photoacoustic method, this study aimed to examine the ethylene changes at different time intervals in the exhaled breath composition of E-cigarette smokers and T-cigarette smokers, before and after the consecutive exposures to cigarettes. Oxidative stress from exposure to tobacco smoke has a role in the pathogenic process, leading to chronic obstructive pulmonary disease. The evidence on the mechanisms by which T-smoking causes damage indicates that there is no risk-free level of exposure to tobacco smoke. The study revealed that the ethylene level (in the E-cigarette smoker’s case) was found to be in smaller concentrations (compared with T-cigarette smoker’s case) and that E-cigarettes may provide an alternative to T-cigarette smoking. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.20.5.051003]

Keywords: CO₂, laser-photoacoustic; electronic cigarettes; traditional cigarettes; breath test; ethylene biomarker.

1 Introduction

Laser photoacoustic spectroscopy (LPAS) is very common now in a wide variety of fields related to physics. From research involving living organisms to air pollution monitoring, spectroscopic gas sensors have proven to be indispensable tools. There are various ways of utilizing gas sensors, and each application has different demands. Some applications require a very high sensitivity for one specific gas compound, while others benefit more from sensors that are able to measure a wide range of gases. A high-time resolution is also desirable, as well as selectivity, robustness, and little or no need for sample preparation.

The E-cigarette closely imitates a T-cigarette since it tastes, looks, and also feels like a traditional one. When “vaping” the E-cigarette, inhaling produces both tactile and craving satisfaction, similar to T-cigarettes, and generates a vaporizing process that releases a vapor mist that evaporates into the air within just a few seconds.

Since the introduction of this product to the consumer marketplace, a number of new companies around the world have started producing them in order to take advantage of the overwhelming positive response being generated by the consumer.2

The question that arises is: Are E-cigarettes much safer than T-cigarettes?

While we cannot make the claim that the E-cigarettes are healthier, we can point out how T-cigarettes are harmful to our health and can put us at higher risk for a whole host of conditions including: stroke, heart attack, lung cancer, throat cancer, pneumonia, osteoporosis, Alzheimer’s, and countless others.2

The relation between ethylene, free radicals, and disease can be explained by the oxidative stress. In a normal healthy human body, the generation of pro-oxidants in the form of reactive oxygen species and reactive nitrogen species is effectively kept in check by the various levels of antioxidant defense. When it gets exposed to adverse physicochemical, environmental, or pathological agents, such as cigarette smoking, atmospheric pollutants, ultraviolet rays, radiation, toxic chemicals, overnutrition, and advanced glycation end products in diabetes, this delicately maintained balance is shifted in favor of pro-oxidants resulting in oxidative stress. It has been implicated in the etiology of several (>100) human diseases and in the process of aging. All the biological molecules present in our body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and even lead to cell death, eventually resulting in diseased states. Membrane lipids present in subcellular organelles are highly susceptible to free radical damage.

When lipids react with free radicals, they can undergo the highly damaging chain reaction of lipid peroxidation (LP), leading to both direct and indirect effects. During LP, a large number of toxic byproducts are also formed that can have effects at a site away from the area of generation, behaving as “second messengers.” The damage caused by LP is highly detrimental to the functioning of the cell. LP is a free radical–mediated process. Initiation of a peroxidative sequence is due to the attack by any species, which can abstract a hydrogen atom from a methyl-ene group (CH₂), leaving behind an unpaired electron on the carbon atom (*CH). The resultant carbon radical is stabilized by molecular rearrangement to produce a conjugated diene, which can then react with an oxygen molecule to produce a
lipid peroxyl radical (LOO∗). These radicals can further abstract hydrogen atoms from other lipid molecules to form lipid hydroperoxides (LOOH) and at the same time further propagate LP. The process of LP gives rise to many products of toxicological interest such as malondialdehyde, 4-hydroxynonenal, and a variety of hydrocarbons including pentane, ethane, and ethylene. Ethylene is a product of the LP of linoleic acid and to assess the free radical damage, photoacoustic (PA) measurement of the exhaled hydrocarbons such as ethylene provides an ideal technique to monitor LP and oxidative stress.3,5 Ethylene from the human breath is a marker of oxidant stress and can be directly attributed to the biochemical events surrounding LP.

Generally speaking, exhaled breath analysis (called breath test) can be represented as follows: production of the biomarker during a particular biochemical reaction or a complex metabolic process; diffusion of biomarker through tissues and input into hematic flow; possible intermediate accumulation (buffering); possible trapping of biomarker by utilization and assimilation systems or natural chemical transformation; transport to the lungs; transmembrane diffusion to the air space of lungs; diffusion of biomarker and their mixing with inhaled air in the alveolar space of lungs; release of biomarker in the breathing air; collection of a breath sample; and assessment of the biomarker in the breath sample.

This paper reports the LPAS as a sensitive, real-time, and noninvasive tool to monitor the concentration of ethylene at E-cigarettes smokers and T-cigarettes smokers at different time intervals.

2 Materials and Method

The CO2 LPAS used for the gas determinations and presented in this report is schematically shown in Fig. 1 and described in detail by Refs. 6 and 7. In brief, PA spectroscopy utilizes a line-tunable CO2 laser and a PA cell where the gas is detected. The requirement for gases to be detected with this sensitive laser is that they should possess a high absorption strength and a characteristic absorption pattern in the wavelength range of the CO2 laser.

Inside the PA cell, traces of ethylene can absorb the laser radiation and the absorbed energy is released into heat, which creates an increase in pressure inside a closed volume. By modulating the laser beam with a mechanical chopper, pressure waves are generated and detected with four microphones of equal sensitivity around the resonance frequency and mounted in the cell wall. The PA signal was measured by a lock-in amplifier with the output filtered data read out by a computer using a data acquisition interface with a TestPoint program, which also reads out the laser power from the power detector via a serial port, controls the chopper frequency, normalizes the data, and automatically stores the files.3,5

CO2 LPAS performs well in terms of sensitive and selective detections of trace ethylene and it allows near online measurements.

The data analysis was conducted for 5 days with 10 male smoker subjects (five of them smokers only of E-cigarettes, and the other five smokers only of T-cigarettes).

To evaluate the breath ethylene, we choose to analyze the effect of the inhalation with E-cigarettes (with 0.5 mg nicotine/drop and 10 mg of nicotine/20 drops) and T-cigarettes (with 0.5 mg/cigarette, 10 mg of nicotine/pack: 0.5 mg×20 cigarettes) at different time intervals (at 9:00 a.m. and 10:00 a.m.) in two sessions.

The subjects were not attempting smoking cessation and were nonalcoholic and nondiabetic, without any chronic mental or physical health problem. Also, the 10 male smoker subjects were asked to avoid coffee and alcohol for at least 6 h prior to their participation in the study and provided three breath samples every day between 8:30 a.m. and 10:00 a.m. (at 8:00 a.m. collection of breath sample before smoking, at 9:00 a.m. collection of breath sample after the first cigarette, and at 10:00 collection of breath sample after the second cigarette inhalation) over a period of 5 days.

The T-cigarette smoker smoked one cigarette/session/0.5-mg nicotine at 9:00 a.m. with 15 to 20 puffs/cigarette and a 10- to 15-s interpuff interval during 10- min smoking session. After a break of about 50 min, the subject starts to smoke the second T-cigarette/session in the second session (used similar conditions to the first cigarettes).

At the same time, the E-cigarette smoker (similar to T-cigarette smoker) puts one drop of 0.5 mg of nicotine E-liquid in the atomizer and starts to inhale with 15 to 20 puffs/drop, a 10- to 15-s interpuff interval and an 10- min “vaping” session (see in the Fig. 4). After a break of about 50 min, each smoker repeated the entire session with one E/T cigarette one more time.

The volunteers were asked to smoke the same brand of cigarette to avoid variability in smoke composition (it is known that the cigarettes from different brands can generate different ethylene levels). Immediately after the final puff of each cigarette, the smoker exhaled in the sample bag (Fig. 2) through the mouth. All the volunteers used the same procedure for inhalation of smoke/vapors from cigarettes.

All the information published about the volunteers (was the subject to their permission) is provided in Table 1.

To get an efficient breath air sample, we used aluminized multipatient collection bags (750-mL aluminum-coated bags), composed of a disposable mouthpiece, a tee-mouthpiece assembly (it includes a plastic tee and a removable one-way flutter valve), and a discard bag for the “dead space” air. Multipatient collection bags (Fig. 2) are designed to collect multiple samples from patients and to hold a sample for a maximum of 6 h.

After an approximately normal inspiration (avoiding filling the lungs to the maximum), the subject places the mouthpiece in his mouth, forming a tight seal around it with the lips, and then normal expiration is made through the mouth in order to empty the lungs of as much air as is required to provide the alveolar sample. The first portion of the expired air (“dead space air”) goes in the discard bag, and the remaining expired air is redirected into the alveolar collection bag. When a suitable sample is collected, the patient stops exhaling and removes the mouthpiece.

After the alveolar air sample is collected, the sample gas is transferred into the PA cell and can be analyzed immediately or

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Fig. 1 Schematic of the CO2 laser photoacoustic spectroscopy (LPAS) instrument.

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Journal of Biomedical Optics

051003-2

May 2015 • Vol. 20(5)
later. In either case, it is recommended to seal the large port with
the collection bag port cap furnished with the collection bag.
The use of the port cap assures that the sample volume will not
be lost due to a leak. Its use also avoids contamination of the
sample by gas diffusion through the one-way valve in the large
port if the sample is stored for a long period of time prior to its
analysis.

To ensure the quality of each measurement, an intensive
cycle of N₂ washing was performed between samples in
order to have a maximum increase of 10% for the background
PA signal. It has to be underlined that the measured PA signal is
due mainly to the absorption of ethylene, but some traces of
CO₂, H₂O, ethanol, etc., influence the measurements (overall
contribution is less than 10%).

To avoid the interference of our molecules of interest with
over 700 species of bacteria that live in our mouths, the subjects
were instructed to use toothpaste and antiseptic mouthwash
before each breath sampling. Also, the response to all absorbing
species at a given laser wavelength (PA signal) decreased con-
siderably when we inserted a KOH trap (with a volume larger
than 100 cm³) proving that the amounts of
CO₂ and H₂O vapors
in the breath can significantly alter the results, making their
removal being compulsory.6,8,9

To analyze the ethylene from the bags, we evacuated the
extra gas and then we flushed the system with pure nitrogen
at atmospheric pressure for few minutes. Then the exhaled air
sample was transferred to the cell at a controlled flow rate of
600 sccm (standard cubic centimeters per minute).

An important parameter in the measurements is the respon-
sivity R (cmV/W) of the PA cell, which depends on the pressure
of the gas inside the cell. Taking into account the fact that the
initial pressure in the sample bags filled by the healthy humans
and by the subjects with different disorders differs from one case
to other, it is necessary to know the pressure dependence of the
PA cell responsivity (Fig. 3). The exhaled air sample was trans-
ferred to the PA cell at a controlled flow rate of 600 sccm, and
the total pressure of the gas in the PA cell was measured, apply-
ing the correction factor for the responsivity according to the
calibration curve from Fig. 3.

The responsivity of the PA cell was determined by using a
calibrated mixture (Linde Gas) of 9.88-ppmV (±2%) C₂H₄
diluted in nitrogen 6.0 (purity 99.9999%) and of 0.96-ppmV
(±5%) C₂H₄ diluted in nitrogen 5.0 (purity 99.999%).6 The
pressure dependence of the responsivity was always measured
at the center of the CO₂ laser line using a frequency-stabilized
laser (instability 3 × 10⁻⁸).

The absorption coefficients of ethylene at different
CO₂ laser wavelengths were previously precisely measured,6 and the
CO₂ laser was kept tuned to the 10P (14) line where ethylene exhibits
a strong peak, corresponding to an absorption coefficient of
30.4 cm⁻¹ atm⁻¹.

### Table 1: Subjects information for T-cigarette smoke and E-cigarette
vapors exposure study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age</th>
<th>Subjects height (m)</th>
<th>Subjects weight (kg)</th>
<th>Smoker since</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Male</td>
<td>23</td>
<td>1.81</td>
<td>73.0</td>
<td>2011 (E-cig.)</td>
</tr>
<tr>
<td>S2</td>
<td>Male</td>
<td>28</td>
<td>1.83</td>
<td>97.0</td>
<td>2010 (E-cig.)</td>
</tr>
<tr>
<td>S3</td>
<td>Male</td>
<td>31</td>
<td>1.62</td>
<td>56.0</td>
<td>2009 (E-cig.)</td>
</tr>
<tr>
<td>S4</td>
<td>Male</td>
<td>29</td>
<td>1.79</td>
<td>78.0</td>
<td>2011 (E-cig.)</td>
</tr>
<tr>
<td>S5</td>
<td>Male</td>
<td>23</td>
<td>1.68</td>
<td>83.0</td>
<td>2011 (E-cig.)</td>
</tr>
<tr>
<td>S6</td>
<td>Male</td>
<td>35</td>
<td>1.78</td>
<td>98.0</td>
<td>2011 (T-cig.)</td>
</tr>
<tr>
<td>S7</td>
<td>Male</td>
<td>32</td>
<td>1.78</td>
<td>81.0</td>
<td>2008 (T-cig.)</td>
</tr>
<tr>
<td>S8</td>
<td>Male</td>
<td>37</td>
<td>1.93</td>
<td>99.0</td>
<td>2007 (T-cig.)</td>
</tr>
<tr>
<td>S9</td>
<td>Male</td>
<td>27</td>
<td>1.65</td>
<td>62.0</td>
<td>2009 (T-cig.)</td>
</tr>
<tr>
<td>S10</td>
<td>Male</td>
<td>28</td>
<td>1.84</td>
<td>73.0</td>
<td>2009 (T-cig.)</td>
</tr>
</tbody>
</table>

![Fig. 2 Breath sample collection system: (a) mouthpiece and tee-connector; (b) 0.75-L aluminum-coated
discard bag; and (c) 0.40-L discard bag.](image-url)

![Fig. 3 The responsivity of the PA cell against the pressure.](image-url)
3 Results and Discussion

Figure 4 shows the average concentrations of breath ethylene for five subjects, before and after exposures to one E-cigarette/session.

Each breath smoker was investigated 5 days with 2 exposure sessions/day and one cigarette/session (with about 50-min break between sessions).

The baseline for E-smokers was: 20 ppb (the breath sample was collected before the exposure to E-cigarette: at 8:30 a.m.), and after the first E-cigarette inhalation/Session 1, the mean ethylene level for Subject 1 (S1) was about 47 ppb, for S2: 45 ppb, for S3: 47 ppb, for S4: 53 ppb, while for S5 in Session 1: 49 ppb.

For Session 2, the values of ethylene concentrations for the exhaled breath samples were: S1–53 ppb, S2–48 ppb, S3–45 ppb, S4–49 ppb, whereas for S5 the value was: 56 ppb.

Figure 5 shows the average concentrations of breathed ethylene for five T-cigarettes smokers, before and after exposures to T-cigarettes.

The baseline for T-smokers was: 27 ppb (before the exposure to T-cigarette at 8:30 a.m.), and immediately after the first T-cigarette inhalation (Session 1), the mean ethylene level increased for S1 to 145 ppb. Following the second T-cigarette inhalation (Session 2), the mean ethylene concentration increased to 187 ppb.

For the other breath samples in Sessions 1 and 2, exhaled ethylene breaths were increased to 149 and 210 ppb for S2, 143 and 185 ppb for S3, 123 and 195 ppb for S4, and for S5 the values are: 154 and 213 ppb.

The results were also compared with the ethylene concentration of a nonsmoker subject (6 ppb).

It should be pointed out that the E-cigarettes volunteers did not receive T-cigarettes and the T-cigarettes volunteers did not receive E-cigarettes.

 Reactive gases, such as those in the smoke, can cause damage and breath ethylene can be a response from the damage of the human lung tissue.

Based on literature data\textsuperscript{10–13} and compared with our results, we hypothesized that the E-cigarettes are safer than T-cigarettes because the ethylene concentration from the breath of E-smokers was found to be smaller at different time intervals (9:00 a.m. and 10:00 a.m.).

In the present study, both the feasibility and the importance of monitoring exhaled ethylene from different subjects have been shown. The ethylene gas, a biomarker of oxidative stress, has been measured using a $\text{CO}_2$ laser-based PA spectrometer.

4 Conclusions

The goal of this study was to determine and verify the evolution of the inhalation of vapors and smoke during $\approx 10$ min of a smoking session over a period of two sessions/two cigarettes (with a 50-min break between sessions) for 10 smoker subjects.

The levels of ethylene trace gas are much lower after the inhalation of E-cigarette smokers with E-cigarettes at different time intervals compared to inhalation of T-cigarette smokers with T-cigarettes at different time intervals.

The results obtained here suggest that the toxic components of T-cigarettes’ smoke are deposited in the lung through
inhalation and this has an effect on the activation of an endogenous source of free radicals and the appearance of oxidative stress together with LP, which leads to inflammatory gene activation. The toxic components of T-cigarettes’ smoke may induce ethylene formation in large quantities. As a complex mixture, tobacco smoke is likely to act through multiple pathways causing disease, and multiple genes may be involved. Ethylene oxide is a chemical product that induces genetic damage and may also affect (but is not limited to) the nervous system.

These results demonstrate that the LPAS is a sensitive, non-invasive, and real-time method to accurately analyze breathed ethylene gas concentrations that possess high absorption strengths and a characteristic absorption pattern in the wavelength range of the CO2 laser.

Acknowledgments

The work has been funded by the Sectoral Operational Programme Human Resources Development 2007–2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132395. Conflict of Interest: The authors declare that they have no conflict of interest.

References


Cristina Popa has a PhD in physics, and since 2006 has worked as a scientist researcher at the National Institute for Laser Plasma and Radiation Physics, Optics and Lasers in Life Sciences, Environment and Manufacturing Group. She has considerable results in laser applications in medicine and biology, and numerous studies on LPAS. She has published more than 23 articles in ISI journals and 2 book chapters.