

MEDICAL PHYSICS

**CO₂ LASER-PHOTOACOUSTIC STUDY OF EXHALED BREATH
PRODUCED BY ELECTRONIC vs. TRADITIONAL CIGARETTES**

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Abstract. CO₂ laser-photoacoustic technology was used to investigate the suitability of ethylene as biomarker for active smoking with electronic cigarettes (E-cigarettes) vs. traditional cigarettes (T-cigarettes). Higher levels of ethylene in the composition of breath after T-cigarettes inhalation was found in comparison with the inhalation with E-cigarettes. The goal of this study was to explore and verify that E-cigarettes are not a potential cause of damage in the human lung tissue at smokers. The CO₂ laser-photoacoustic spectroscopy is suitable for the detection of ethylene in exhaled breath, producing feasible and reproducible results which discriminate active smoking with E-cigarettes vs. T-cigarettes. This study is probably the first measurement of the ethylene response from the damage of the human lung tissue at exhaled breath of E-cigarettes smokers. Exhaled breath air analysis using CO₂ laser-photoacoustic spectroscopy is a non-invasive method, safe, rapid and acceptable to subjects, with promising potential in monitoring and diagnostics of ethylene patterns from active smoking vs. healthy breath samples.

Key words: laser photoacoustic spectroscopy, active electronic smoking, active traditional smoking, breath ethylene.

1. INTRODUCTION

Smoking is still the leading cause of avoidable death of an estimated five million people worldwide every year. It is the second leading cause of death, after cancer, and causes 42% of cases of chronic respiratory disease (including asthma, bronchitis and emphysema), 10% of cardiovascular diseases (including heart disease and stroke), increased risk of Alzheimer’s disease (developing by 0.7 %), and increased risk of developing age-related macular degeneration [1].

People who smoke are not only hurting themselves, but they also can harm non-smokers. The World Health Organization estimates that second hand smoke kills six hundred thousand people each year, while expectant mothers who smoke are more likely to have babies with health problems and low birth weight [1, 2].

In recent years, cigarette smoking has been legally banned in many public places. Since then, smokers have been searching for smoking alternatives to help curb their nicotine cravings. Some people wear a nicotine patch, some people chew nicotine gum, and some people switch to E-cigarettes.

One of the main differences in tobacco products and E-cigarettes is that E-cigarettes do not produce smoke or tar. When you smoke a tobacco cigarette, smoke is expelled into the air. This smoke contains over 4,000 different pollutants and toxins. When you smoke an E-cigarette, you are inhaling and exhaling vapors that contain nicotine, but they contain no pollutants and no second hand smoke is released, so the passive smoking danger is avoided [3–5].

Although E-cigarettes have recently been marketed as a safer alternative to traditional smokes, a new analysis of 19 types of E-cigarettes revealed that they contain toxic chemicals [1–5].

The purpose of this study was to evaluate traces of ethylene in exhaled breath air resulting from lipid oxidation in lung epithelium following the inhalation of cigarette smoke and “electronic vapors” using CO₂ laser-photoacoustic technique.

The cigarette smoke contains many toxic components that may induce ethylene formation. Ethylene oxide is a chemical product that induces cancer in the lungs, genetic damage and may also affect nervous system. To monitor the damages caused by the inhaled smoke and inhaled vapor, a breath test was performed in order to obtain information on the ethylene concentrations under different circumstances.

We compared exhaled ethylene breath samples from subjects who inhaled T-cigarette smoke and subjects who inhaled E-cigarette smoke.

The results presented here have shown that there are significant differences between the composition of breath produced by E-cigarettes and composition of breath produced by T-cigarettes.

2. METHOD AND MATERIALS

The CO₂ laser-photoacoustic spectroscopy instrument used for the experiment presented in this study is schematically shown in Figure 1 and was described in detail elsewhere [6–9]. In brief, photoacoustic spectroscopy utilizes a line-tunable CO₂ laser and a photoacoustic cell, where the gas is detected. The requirement for gases to be detected with this sensitive laser instrument is that they should possess high absorption strength and a characteristic absorption pattern in the wavelength range of the CO₂ laser.

Inside the photoacoustic 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 with equal

sensitivity around the resonance frequency and mounted in the cell wall. The photoacoustic signal was measured by a lock-in amplifier using a time constant of 1 s. The output filtered data of the lock-in amplifier were 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 data and automatically stores files [6].

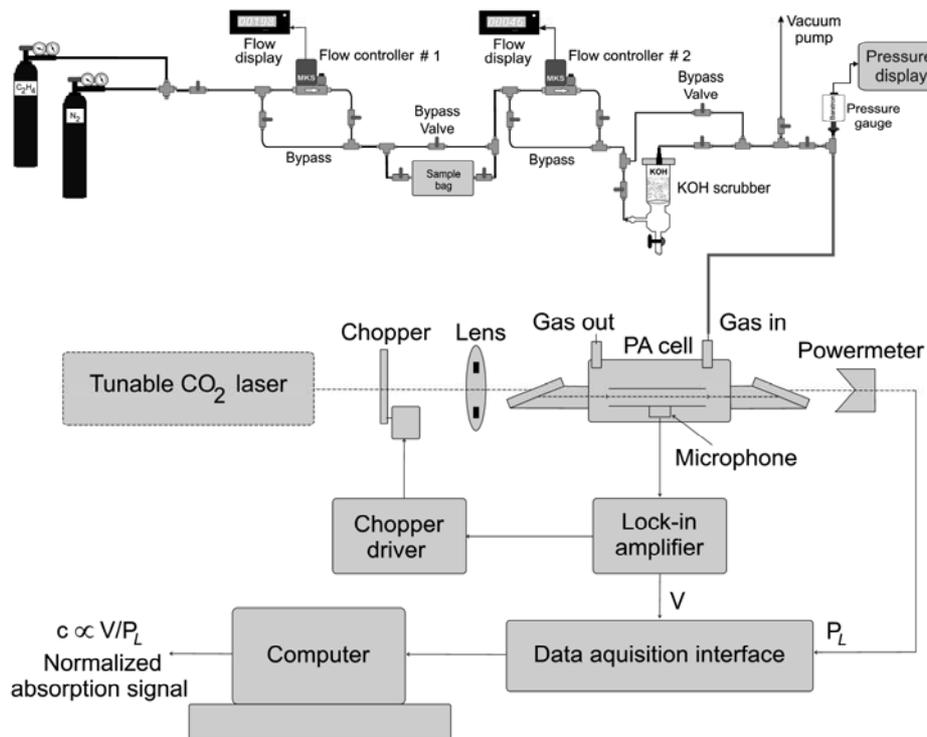


Fig. 1 – Schematic of the CO₂ laser-photoacoustic spectroscopy instrument.

Another essential element in these measurements is the gas handling system due to its role in ensuring gas purity in the photoacoustic cell. For cell response calibration, a certified mixture of 9.88 ppmV ($\pm 2\%$) ethylene in pure nitrogen 6.0 (purity 99.9999%) (Linde Gas) was used.

With CO₂ laser-photoacoustic spectroscopy, ethylene can be detected in near real time, with high sensitivity, high speed and very good selectivity.

The measurements, which were conducted with two smoker subjects and one non-smoker subject (considered healthy), were done to evaluate the effect in near real time of the inhalation with E-cigarettes vs. T-cigarettes associated with

ethylene biomarker in the exhaled breath of each smoker. The volunteer subjects (two males and one female, with ages between 27 and 30 years) were included in this analysis. In addition, some smoker participants ($n = 3$) were withdrawn from the experiment due to failure to comply with study procedures. The participants were isolated in a room with area of 55 m² and volume of 190 m³ in which experiments were conducted at room temperature (23–25 °C).

Before inhalation of the first E-cigarette smoke (10 mg of nicotine E-liquid with 0.5 mg per drop) or T-cigarette (10 mg of nicotine per pack with 0.5 mg per cigarette), the smoker subject provided ethylene baseline sample (background breath concentration) by breathing only unfiltered room air and exhaling *via* a disposable mouthpiece into aluminized multi-patient collection bag (750 ml).

The investigated subjects were not in the stage of smoking cessation attempt, were non-alcoholic and non-diabetic, without any chronic mental or physical health problem. Also the subjects were asked to abstain from cigarette smoking, to avoid coffee and alcohol for at least 10 hours prior to their participation in the study.

The subjects also responded to a medical history questionnaire with: general information; past medical history – past surgery, drug reactions/allergies, diagnostic studies; social/lifestyle history and family medical history.

Each participant smoked one T-cigarette per session, with 15–20 puffs per cigarette and 10–15 seconds interpuff interval, during a 5 min to 10 min *ad libitum* smoking period. After 15 min interval between ending one cigarette and beginning another, each participant smoked one E-cigarette (used similarly to a T-cigarette). After a break of about 15–20 min, the smokers repeated the entire session (exposure session during a 30 to 40 min), three more times. Also, smokers were free to smoke their usual brand of cigarette to avoid variability in smoke composition.

The inhalation with T-cigarette *vs.* E-cigarette was initiated by each smoker and immediately after the final puff the smoker places the mouthpiece in his mouth, forming a tight seal around it with the lips and a normal expiration (avoiding filling the lungs at maximum) is then made through the mouth, in order to empty the lungs of as much air as required to provide the alveolar sample. The first portion of the expired air goes out and the remaining expired air is redirected into the collection bag. When a suitable sample is collected, the participant stops exhaling and remove the mouthpiece.

All the data about smoker participants are published here with their permission.

3. RESULTS AND DISCUSSIONS

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. This analysis focuses on the ethylene concentration changes in the exhaled breath composition of smokers' breath immediately after the exposure with T-cigarettes and E-cigarettes.

The subjects were asked to exhale into sample bags at a normal exhalation flow rate. To analyze the bags contents, firstly we evacuate the extra gas by the vacuum handling system, and then we flushed the system with pure nitrogen at atmospheric pressure for 30 minutes. The exhaled air sample was transferred to the cell at a controlled flow rate of 600 sccm (standard cubic centimeters per minute), and the total pressure of the gas in the photoacoustic cell was measured.

During the transfer of the exhaled air from the collecting bag to the cell, the sample gas was passed through a trap filled with KOH pellets to remove the CO₂ and H₂O. When this trap with a volume larger than 100 cm³ was inserted, we found out that the signal (the response to all absorbing species at a given laser wavelength) decreased considerably, showing that the existing amounts of CO₂ and H₂O in breath can alter significantly the results, thus their removal being compulsory [10–12].

The information on the participants for ethylene breath test protocol is provided in Table 1.

Table 1

Information on subjects for T-cigarettes smoke and E-cigarettes vapors exposure study

Subject	Gender	Age	Subjects height (m)	Subject weight (kg)	Smoker since
P1	Male	29	1.83	77.0	2008
P2	Male	28	1.77	99.0	2008
P3	Female	30	1.60	50	–

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

To observe the impact of E-cigarettes, we chose to expose the smokers first to T-cigarettes (smoke) and then to E-cigarettes (vapors). Each smoker was investigated for 10 days, with 3 exposure sessions per day. Figure 2 shows the average concentrations of breath ethylene before (baseline) and after exposure to cigarettes for P1 and P2.

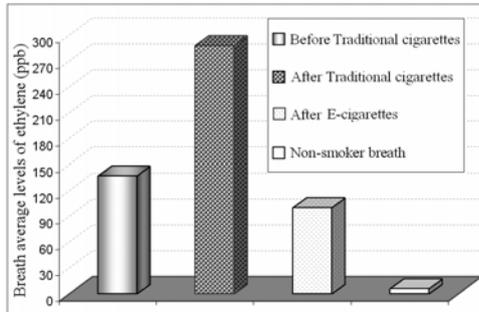


Fig. 2 – a) Breath average levels of ethylene for smoker 1 with T-cigarettes vs. E-cigarettes.

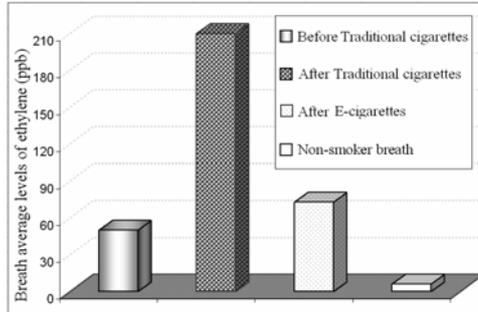


Fig. 2 – b) Breath average levels of ethylene for smoker 2 with T-cigarettes vs. E-cigarettes.

As an observation of our primary result of interest (shown in Figure 2), we see that immediately after T-cigarettes inhalation, the mean ethylene level is about 300 ppb for smoker 1 and 210 ppb for smoker 2, respectively. When the T-cigarette was changed with an E-cigarette, the mean ethylene level became smaller compared to that of T-cigarette (100 ppb for smoker 1 and 73 ppb for smoker 2, respectively).

Then, to observe the impact of T-cigarettes, we exposed the subjects first to vapors (E-cigarettes) and then to smoke (T-cigarettes). Each smoker was investigated for 6 days, with 3 exposure sessions per day. Figure 3 shows the average concentrations of breath ethylene before and after exposure to cigarettes for P1 and P2.

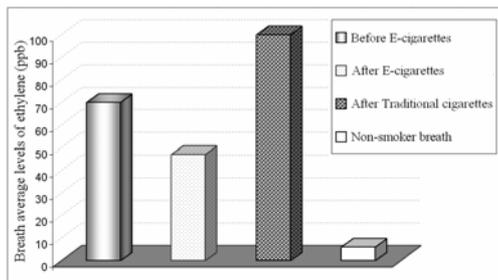


Fig. 3 – a) Breath average levels of ethylene for smoker 1 with E-cigarettes vs. T-cigarettes.

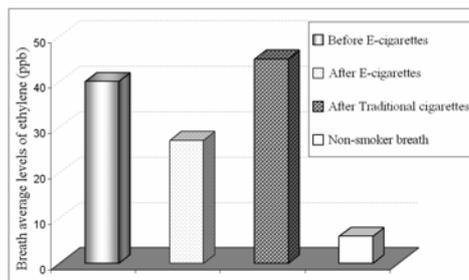


Fig. 3 – b) Breath average levels of ethylene for smoker 2 with E-cigarettes vs. T-cigarettes.

The concentration of ethylene from each smoker exposed to vapors was reduced (smoker 1: 47 ppb; smoker 2: 27 ppb). When the vapors were changed with smoke, we observed the impact of T-cigarettes for each smoker (smoker 1: 100 ppb; smoker 2: 45 ppb, approximately double values).

For both experiments, we tested the capacity of the CO₂ laser-photoacoustic spectroscopy technique to distinguish the subjects assumed to be non-smoker. In this case it should be pointed out that the non-smoker participant did not receive E-cigarettes or T-cigarettes.

Active smoking of T-cigarettes contributes to cause different diseases (like cancer and cardiovascular diseases). The toxic components of T-cigarettes smoke are deposited in the lungs and have as effect activation of endogenous source of free radicals and appearance of oxidative stress together with lipid peroxidation, which leads to inflammatory gene activation. As a complex mixture, tobacco smoke is likely to act through multiple pathways in causing disease, and multiple genes may be involved.

Based on literature data [5, 13, and 14] and compared with our results, we hypothesized that E-cigarettes are safer than T-cigarettes.

4. CONCLUSIONS

The purpose of this study was to determine if E-cigarettes can cause damage as T-cigarettes. We compared the level of ethylene from smoker's breath air when they are exposed to vapors or to smoke.

Reactive gases in the smoke can cause damage and ethylene can be a response from the damage of the human lung tissue.

In the first case, when the T-cigarette is changed by an E-cigarette, the mean concentration of breath ethylene decreases by about 35%, while in the second case, when the E-cigarette is changed by a T-cigarette, the mean concentration of breath ethylene increases by about 50%.

The results obtained here give the useful information that smoking T-cigarettes, which release in tobacco smoke a complex chemical mixture of combustion compounds (like burned nicotine and tar), causes adverse health outcomes, particularly cancer, cardiovascular and pulmonary diseases, through mechanisms that include DNA damage, inflammation, and oxidative stress.

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 (lipid peroxidation) by which T-smoking causes disease indicates that there is no risk free level of exposure to tobacco smoke.

E-cigarettes (where nicotine is released into vapors) may help reduce smokers' exposure to toxins. Nicotine (while is a highly addictive substance), is not what causes cancer for smokers or for the people around them who breath their second hand smoke.

In summary, the study revealed that E-cigarettes are not so dangerous to cause damage at smokers, because the ethylene in this case was found to be in

smaller concentrations. That is why E-cigarettes may provide an alternative or a substitute to T-cigarettes smoking.

This analysis is the first study to evaluate the damage of ethylene at exhaled breath of E-cigarettes smokers vs. T-cigarettes smokers.

The measurements presented here confirm that the analysis of smokers exhaled breath with CO₂ laser-photoacoustic spectroscopy based instruments is a reliable and non-invasive method, with potential in monitoring the ethylene biomarker from active smoking breath samples.

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