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**Solid matter and soluble compounds collected from cigarette smoke and heated tobacco product aerosol using a laboratory designed puffing setup**

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## Abstract

A laboratory setup recently designed has been used to perform puffing experiments in conditions similar to those of Health Canada Intense regime with the purpose of collecting and studying any particulate and/or soluble matter generated as a result of cigarette smoking or Heets use in an IQOS device. Smoke or aerosol can leave deposited matter in several parts of the setup, roughly resembling the interaction with the human body. Samples have been collected from different parts of the setup. For cigarettes, the extracted solutions were yellowish, whereas they remained colourless for Heets. This indicates that the content of both the deposited particulate matter and the amount soluble compounds were much higher in cigarettes smoke than in Heets aerosol. Not only quantitative differences have been found. Thus, the solid matter collected from cigarettes smoke contains some insoluble fractions mainly composed by C and O, but also by traces of S, K, Ca, Fe, As, Na, Al, Si, and Ba, while the analogous samples from Heets are mainly composed of C and O and are soluble in isopropanol. In addition, in Heets aerosol a relatively low fraction of the detected compounds corresponds to polycyclic aromatic hydrocarbons (PAHs), compared to the percentage of PAHs present in the cigarette smoke. When cigarettes were smoked under a continuous smoking regime (continuous air flow) solid matter was found to be deposited on a part of the setup. This collected solid matter was composed mainly of C and O (being mostly insoluble in water and partially soluble in isopropanol) and contained traces of heavy metals (As, Cd, Cr, Ni, Cu, and Pb).

## Keywords

Heated tobacco products, cigarettes, particulate matter, collection.

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## 1. Introduction

The presence of harmful and potentially harmful constituents in the smoke resulting from burning tobacco is well known. There is a strong connection between the toxicity of tobacco smoke and lung cancer risk, heart disease, emphysema, and other serious diseases [1,2]. Indeed, particles and droplets generated in tobacco smoking contain more than 3500 semi-volatile and non-volatile compounds, some of which are classified as carcinogenic, such as polycyclic aromatic hydrocarbons (PAHs) [3], dioxins and furans [4], heavy metals [5], and tobacco-specific nitrosamines [6].

Recently, new nicotine delivery gadgets have been developed as alternatives to tobacco smoking to avoid the intake of compounds generated in the combustion of traditional cigarettes. In this sense, IQOS is a heated tobacco product commercialized by Phillip Morris International (PMI) in 2014 [7], in which tobacco sticks (called Heets) are electrically heated at a temperatures up to about 350 °C to generate an aerosol upon inhalation, but too low to produce the tobacco combustion [8,9]. As combustion does not occur, and therefore combustion-related chemicals are not generated, from that point of view this system is expected to be less harmful than regular cigarettes.

Due to its recent commercialization, there are very few studies aiming to characterize the aerosol generated by the IQOS and, particularly, the potential deposits that it can leave behind. Thus, Mitova et al. [10] have compared the concentrations of representative particulate and vapor phase constituents present in the mainstream cigarette smoke and IQOS aerosol when used in an environmentally controlled room. They have shown that the composition of the indoor air after IQOS use is similar to that of the background levels determined when panelists not using any tobacco product were present. Only acetaldehyde and nicotine concentrations were slightly higher than the background concentrations. Similarly, Ruprecht et al. [11] have compared second-hand emissions from traditional cigarettes and IQOS use focusing on particulate metals and organic compounds by conducting continuous and time-integrated measurements in an indoor environment. They found that polycyclic aromatic compounds (PACs) and heavy metals are almost non-detected in the IQOS aerosol, whereas certain n-alkanes and organic acids are emitted in substantial levels (up to 9236 in front of 11453 ng/h as part of cigarette smoke). They have also proved that the emission rate of sulfur during IQOS and cigarette consumption is 3729 and 34540 ng/h, respectively). Simonavicius et al., after their

systematic literature review on second-hand emissions studies concluded that, compared with nicotine in smoke of conventional cigarettes, in mainstream IQOS aerosol nicotine ranged from 57% to 83% respect to conventional cigarettes, and levels of harmful and potentially harmful toxicants were reduced by at least 62% and of particulate matter by, at least, 75% [12]. Davis et al. [13] and Bekki et al. [14] have compared the concentration of harmful compounds in the mainstream and fillers of IQOS sticks and conventional combustion cigarettes. From IQOS, they recognized a nicotine content similar to conventional cigarettes, and a reduced, but not negligible, emission of tobacco-specific nitrosamines. Pacitto et al. [9] have found that particle concentration in the IQOS mainstream aerosols (including both volatile and non-volatile particles) is less than  $1 \times 10^8$  part.  $\text{cm}^{-3}$ , lower than in smoke from traditional tobacco ( $3.14 \times 10^9$  part.  $\text{cm}^{-3}$  [15]), and that the corresponding mean particle is roughly around 100 nm, smaller than in the tobacco cigarette smoke (150-200 nm).

In a previous study, an experimental laboratory setup has been designed to trap in liquid media (i.e. water or any other solvent) any particulate matter or soluble compounds present in the cigarette smoke or in the IQOS aerosol [16], inspired by the work of Horváth et al [17]. Briefly, in such a setup, the smoke or the aerosol is driven with aid of a vacuum pump through a liquid bed inside a gas washing flask where small bubbles are formed thanks to a holey rubber piece capping the down-comer glass tube (Figure 1). Particulate matter and condensable compounds could be deposited along the smoke or aerosol pathway or trapped in the liquid (either as solid particles or dissolved compounds). The mentioned previous work [16] has focused on the study of trapped species in the liquid contained in the gas washing flask, either water (resembling the interaction of smoke or aerosol with the hydrated biological tissues) or isopropanol (a good solvent of organic compounds). In the previous study, the information from several techniques (total organic carbon analysis, laser diffraction, UV-vis spectrophotometry and transmission electron microscopy) has shown that cigarette smoke contains a large number and amount of compounds soluble in water and isopropanol, together with solid particles generated during combustion. In contrast, negligible presence of non-combustion related particles was detected in Heets aerosol samples. Nevertheless, the analysis, characterization, and quantification of solid matter deposited on other parts of the experimental setup (parts (1)-(3) in Figure 1) would give a useful complementary information about the emissions involved in cigarettes smoking and Heets use. Deposit

of solid matter on surfaces along the smoke or aerosol pathway would have some resemblance with the effects of the circulation of these streams in parts of the body such as mouth and pharynx.

In the present study, samples collected from different parts of the setup have been quantitatively determined and thoroughly characterized using several experimental techniques.

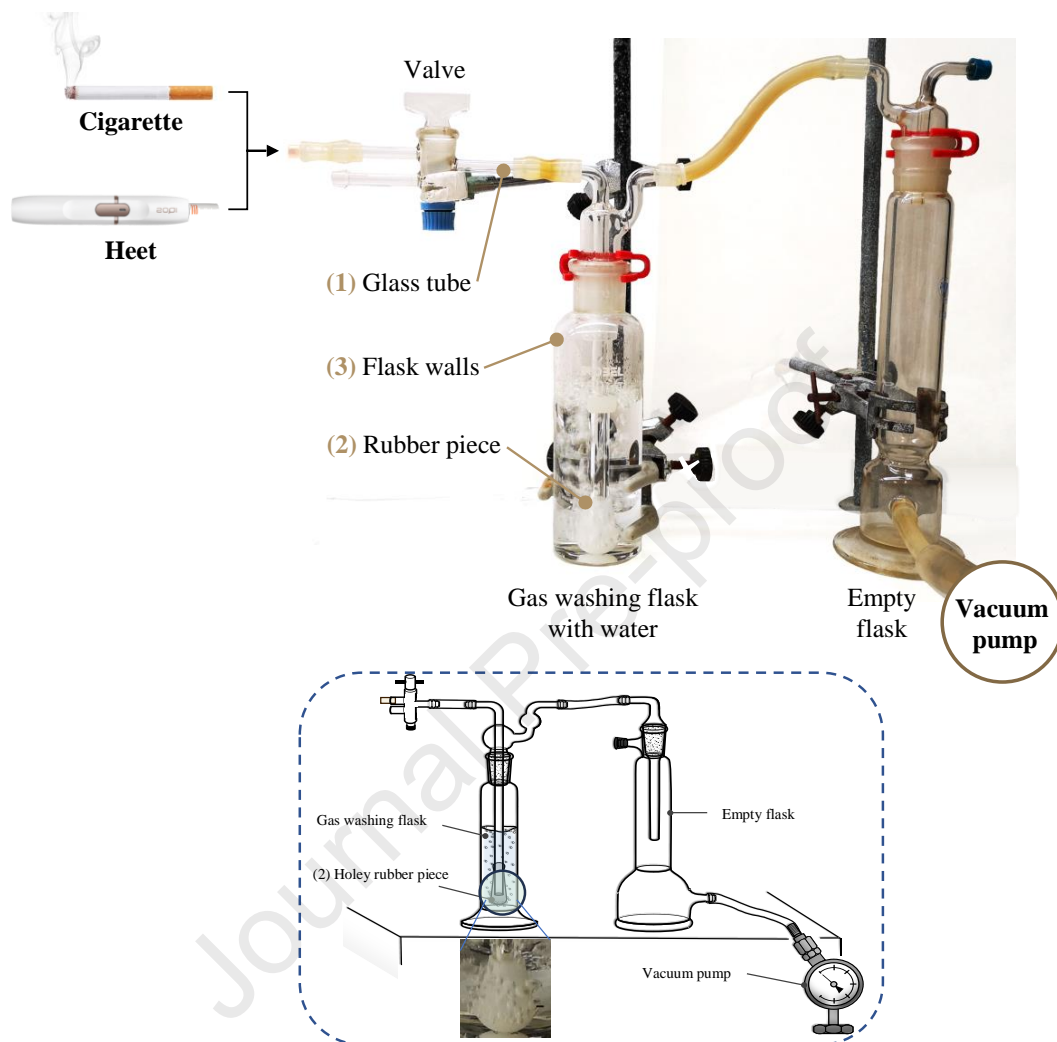
## 2. Materials and methods

### 2.1. Description of smoking tests

Smoking tests have been carried out with the following tobacco products, purchased in a local tobacco shop in Alicante: regular tobacco cigarettes (Marlboro Red Medium, Philip Morris USA Inc., Richmond, VA) and Heets (amber box from Philip Morris Products S.A., Neuchâtel, Switzerland) with the use of the IQOS device (2.2 model, supplied by Philip Morris Products S.A. (Neuchâtel, Switzerland)). According to other researchers, the nicotine and tar contents in Marlboro Red Medium are 0.8 mg/cig and 10 mg/cig, respectively [18]. The nicotine content reported for Heets (amber box) is 0.5 mg/Heet [19]. Determination of tar is not applicable to heated tobacco product aerosols as there is no combustion.

The experimental setup, designed and optimized in a previous study [16], is shown in Figure 1, where the main components are identified. The experiment starts by lighting the cigarette, or switching on the IQOS, and simultaneously switching on the vacuum pump. This extraction simulates the inhalation, and the generated smoke or aerosol (from a cigarette or a Heet) is driven through the water bed inside a gas-washing flask in the form of small bubbles (thanks to a holey rubber piece (2)). The smoke (or the aerosol) may leave some solid deposits in its way, firstly on the glass tube (1), then on the holey rubber piece (2) and, finally, on the flask walls (3) (after having passed through the water bed inside the gas washing flask, where some solid matter and soluble compounds could also become trapped). Water-trapped substances have been studied in a previous work [16], mainly focused on the characterization of any particulate matter retained in the gas washing flask filled with water or isopropanol and on the comparison of the results when cigarettes or Heets were used. The obtained results revealed that aerosol samples from

IQOS were almost free from particulate matter, whereas the solutions obtained after smoking cigarettes clearly showed particles and were colored [16].



**Figure 1.** Photograph and scheme (dotted) of the experimental setup to simulate cigarette smoking or Heets use. Any solid deposits in parts numbered 1, 2, and 3 have been collected. The holey rubber piece was “designed” so as to maximize the contact time between aerosol and water, and it can be observed in the dotted setup scheme and photograph.

\* The vacuum pump forces the aerosols to pass to the experimental step up and through the water. Hence, any particulate matter and/or organic compound are collected inside in a closed system, the setup (either collected in water or on the surface of the closed setup).

The present work focuses on the study of any solid mater (apart from that being trapped in liquid) deposited during puffing experiments in some selected parts of the experimental setup (see Figure 1). In addition, and to get the most accurate information, not only experiments with puffing, but also new tests without simulating puffs



(intensive regime) have been carried out in this work. Thus, the present study analyses, in an exhaustive way, any particulate matter deposited on the rubber piece, on the flask walls, and on the glass tube and would simulate any particulate matter deposition in parts of the body such as the mouth and pharynx, and others. It is interesting, and complementary, to investigate and study both types of interactions.

Thus, in the present investigation simulated puffs have been performed in conditions according to the Health Canada Intense regime [20] (inhalation of a 55 mL volume in 2 s every 30 s) with the help of a glass stopcock, identified as “valve” in Figure 1. Puffing experiments have been performed using 15 cigarettes or 15 Heets. Continuous flow experiments (non-puffing tests) using 15 cigarettes have been also carried out. Table 1 compiles a summary of the experiments performed, including the average time of a cigarette or Heet consumption and the number of puffs required in each case.

**Table 1.** Consumption time per cigarette/Heet and number of puffs performed. The results have been obtained as an average of 6 experiments.

Entry	Simulating puffs	Number of cigarettes	Number of Heets	Consumption time per cigarette/Heet (min)	Number of puffs per cigarette/Heet
1	No	15	-	2.6	-
2	Yes	5	-	4.5	11
3	Yes	15	-	5.8	11
4	Yes	-	5	5.8	11
5	Yes	-	15	5.8	11

## 2.2. Sample collection and characterization of collected samples

In order to fully characterize what is generated during tobacco smoking and Heets use, sampling in different parts of the setup has been performed. Thus, a fixed protocol for samples sampling and analysis has been established and followed to ensure the quality and reproducibility of the experiments detailed in Table 1 (entries 1-5).

Each experiment was repeated at least five times and the different samples were collected using the protocol described below.












For each of the five repetitions of the experiment in **entry 1**, corresponding to 15 cigarettes smoked with a continuous air flow, a brown viscous material was deposited on the rubber piece of the setup (part 2 in [Figure 1](#)), and it was collected using a spatula. The average mass of collected solid per experiment was 0.2 g, which corresponds to 13.3 mg viscous material per cigarette (these are average values obtained from the five repeated tests, 15 smoked cigarettes each).

Such a brown viscous material was not deposited on the rubber piece of the setup in any of the five repetitions of puffing experiments (**entries 2-5**).

The procedure for the collection of solid matter on the glass tube (part 1) or flask walls (part 3) after continuous flow (**entry 1**) or puffing experiments (**entries 2-5**) was standardized as follows: isopropanol (5 mL) was put in contact with the specific setup part to allow the solvent to drag any potential soluble and insoluble compounds. The process was repeated 8 times in each part and, thus, 8 aliquots of 5 mL of isopropanol were collected. Thus a total volume of solution of 40 mL was available for analysis. The procedure was fixed after having checked that more than 8 times treatment using 5 mL did not lead to any additional collection of compounds.

[Table 2](#) shows images of these collected samples. In experiments involving cigarettes, the isopropanol solution becomes yellowish, whereas it remains colourless in the case of experiments performed with Heets.

**Table 2.** Photographs of the collected samples from each part of the setup (see [Figure 1](#)).

Type of tests		15 cigarettes without puffs	5 cigarettes with puffs	15 cigarettes with puffs	5 Heets with puffs	15 Heets with puffs
Setup part	Glass tube* (1)					
	Rubber piece (2)		Solid not observed	Solid not observed	Solid not observed	Solid not observed
	Flask walls* (3)					

\* Samples collected using 40 mL isopropanol.

The liquids were directly analyzed, whereas different analysis approaches were used for the brown viscous collected material. In any case, the following characterization techniques have been used as described:

- Thermogravimetry (TG) (TG-DSC2, Mettler-Toledo) in air flow (100 mL/min) at 5 °C/min up to 120 °C, 1 h soaking time, followed by heating at 5 °C/min up to 800 °C, or in N<sub>2</sub> flow (100 mL/min at 5°C/min up to 800°C). Temperature programmed desorption (TPD), using a DSC-TGA (TA Instruments, SDT 2960 Simultaneous) coupled to a mass spectrometer (Thermostar, Balzers, GSD 300 T3), in the following conditions: 100 mL/min He, at 10 °C/min up to 950 °C. In this analysis, for each characterization test, two representative portions of the brown viscous collected material were analysed.
- X-ray fluorescence (XRF) using a sequential X-ray spectrometer Philips Magix Pro fitted with a Rh tube and Be window. In this analysis, three representative portions of the brown viscous collected material were analysed.

- Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x). For these measurements, the solid sample was digested in 3.5 mL HNO<sub>3</sub> and 0.5 mL H<sub>2</sub>O<sub>2</sub> (at 240 °C for 15 min). Two representative portions of the collected brown viscous material were analysed by this procedure.
- High resolution transmission electron microscopy (HR-TEM) was performed in a Thermo Scientific TALOS F200x model with energy dispersive X-ray (EDX) spectroscopy signal detection. The solid sample collected on the rubber piece (part 1) was suspended in a liquid and ultrasonically dispersed. Then, a drop of this suspension was deposited on the copper grid (Lacey Carbon Supported Copper Grids 200 mesh) and evaporated. Two solid samples were analysed by this method. In the case of the liquid samples collected from parts 1 and 3, the grid was immersed in them and then, the solvent was evaporated. Two liquid samples from each experiment were analysed in these conditions. Particle size distribution was determined using the Jmol software and measuring the diameter of, at least, 100 particles.
- UV-Vis spectrophotometry (UV-vis V-670 JASCO). The liquid samples were previously filtered using a 0.2 µm filter (Chromafil®PET-20/25). Two liquid samples from each experiment were analysed in these conditions.
- GC-MS analysis was carried out in a low-resolution mass spectrometer with quadrupole analyser (Agilent 5975C) coupled to a gas chromatograph for capillary columns with split/splitless injector (Agilent 7890A). Two liquid samples from each experiment were analysed in these conditions by direct injection. Injection: splitless, 1 min. A column clean-up procedure was applied between samples, consisting of holding the column temperature at 250°C for 10 min after each experiment.
- XRD analysis (SEIFERT 2002) using Cu Kα (1.54 Å) radiation, in the 6-80° 2θ range and scanning velocity of 2°/min has been used to characterize the ashes obtained from the combustion of the solid matter in air. Ashes of two samples were analysed.

To summarize, note that the brown viscous collected material was directly characterized by TG, XRD, and XRF. In the case of TG, this technique was used to study the nature of the viscous material collected from the rubber piece. The combined use of both analyses

carried out in an inert atmosphere (nitrogen) or air, makes possible to investigate the composition of this collected solid in terms of volatile + semi-volatile matter and ashes.

Treatment in air flow (100 mL/min) at 5 °C/min up to 120 °C, 1 h soaking time, followed by heating at 5 °C/min up to 800 °C, was performed to quantify the ash content [21], and treatment in N<sub>2</sub> flow (100 mL/min at 5°C/min up to 800°C) was carried out to quantify the volatile and semi-volatile matter [21].

Duplicates were measured in all the characterization experiments of this study to ensure validity and reproducibility.

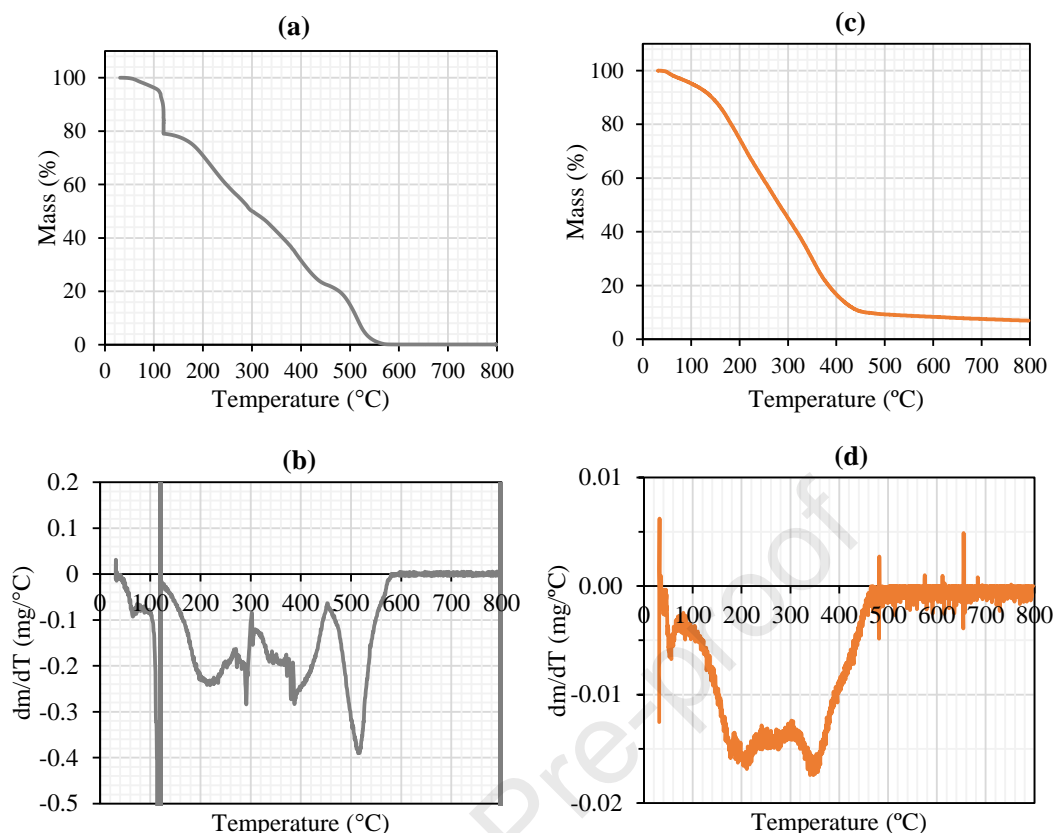
### 3. Results and Discussion

The obtained results are organized considering the parts of the setup where the samples were collected.

#### 3.1. Solid deposited on the rubber piece (part 2 of the experimental setup)

##### 3.1.1. Direct characterization of the viscous collected matter

Figures 2a and 2b show, respectively, the thermogravimetry and derived-thermogravimetry (DTG) curves obtained in the air flow heat treatment, where several steps can be observed, meaning a non-homogeneous composition of the substance. There is a very small ash residue that accounts 0.1 wt. %. Analogously, Figures 2c and 2d show, respectively, the TG and DTG curves obtained in the experiment carried out in N<sub>2</sub> flow. This experiment has revealed that about 82 wt. % of the sample decomposes, and a thermally stable residue remains (18 wt. %). The mass loss in the inert atmosphere shows a smooth variation (much lower variations in DTG curve, be aware of the scale).



**Figure 2.** Thermogravimetry analysis in air (a and b) and in N<sub>2</sub> (c and d) atmospheres of the solid matter deposited on the rubber piece after 15 cigarettes smoked in continuous flow test.

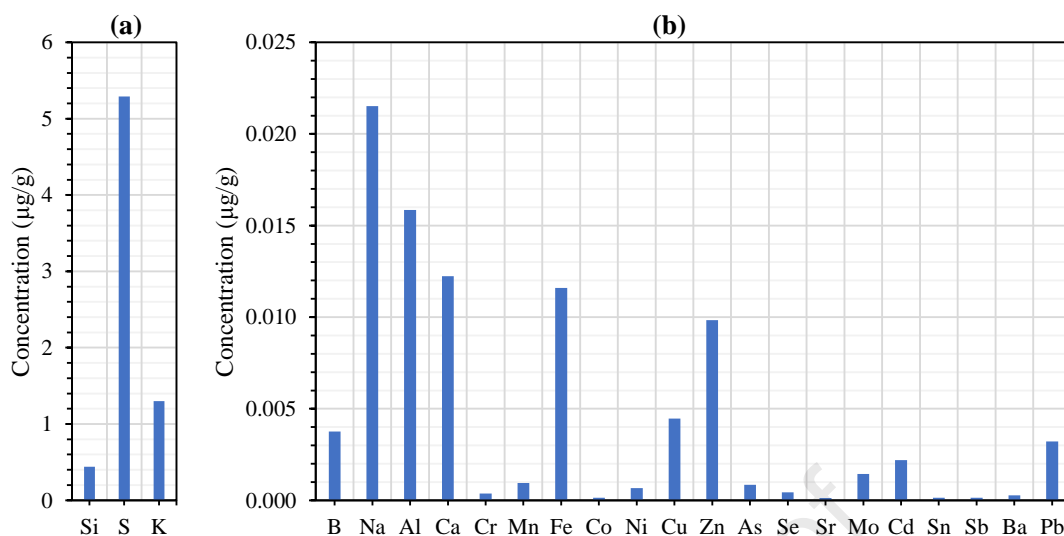
The TG data show that the 13.3 mg of solid matter deposited per cigarette contained approximately 11 mg of volatile and semi-volatile matter, and 13.3  $\mu$ g of ashes. The ash residue was analysed by XRD revealing that it is amorphous (Figure S1, supplementary information).

The XRF analysis of the brown viscous collected material shows that it contains the following elements: S, Ca, K, As, and Fe. Of this set of elements, most of it is S (38.8 wt. %), followed by Ca, K and As (26.1, 16.9, and 15.4 wt. %, respectively), while Fe is found in very small quantities (2.7 wt. %). Two samples obtained in different smoking experiments (with 15 cigarettes each) have been analysed, showing reproducible data. These elements and content percentages are similar to those reported by Paoletti et al. in the XRF analysis of smoke compounds retained in a filter. Other authors have detected traces of S, Na, K, Cl, and Ca and of heavy metals, such as Ni, Co, Zn, Cd, and As in tobacco smoke in ng amounts [22,23].

### 3.1.2. Characterization of digested and dispersed/dissolved samples

Analysis via microwave digestion-ICP-MS of the solid matter revealed that S, K, and Si are the most abundant elements. Other elements, in much lower concentration, were also detected (see Figures 3a and 3b, be aware of the different scales). All the detected elements by XRF are also detected by ICP-MS, although in different proportions. S is the most abundant detected element in both techniques.

Metals most commonly associated with health effects are As, Cd, Cr, Ni, Cu, and Pb [24]. All of them have been detected in low amounts in the digested solid matter collected from cigarettes tests ( $4.46 \times 10^{-3}$  µg/g of Cu,  $3.22 \times 10^{-3}$  µg/g of Pb,  $2.20 \times 10^{-3}$  µg/g of Cd,  $0.85 \times 10^{-3}$  µg/g of As,  $0.67 \times 10^{-3}$  µg/g of Ni and  $0.38 \times 10^{-3}$  µg/g of Cr). Other authors have also found, by ICP-MS, heavy metals in tobacco composition and in the mainstream cigarette smoke. Pinto et al. [24] have reported that Cd, Pb, and As content in tobacco range from 0.14 to 0.79 µg/g. Wang et al. [25] have shown that the heavy metals content in the particulate matter from the mainstream cigarette smoke is almost 0.5 µg/g for Cd, 0.4 µg/g for Pb, 0.2 µg/g for Cr, and lower than 0.1 µg/g for As. In the solid matter collected from cigarettes' tests, the concentration of these metals is two orders of magnitude lower (Figure 3), probably because some amount of the detected metals in the mainstream cigarette smoke could be trapped in other parts of the experimental setup. Note that this statement does not mean uncontrolled deposition in any part of the setup. It means that the metals analyzed are not fully collected in the part of the setup which is the object of that particular analysis.

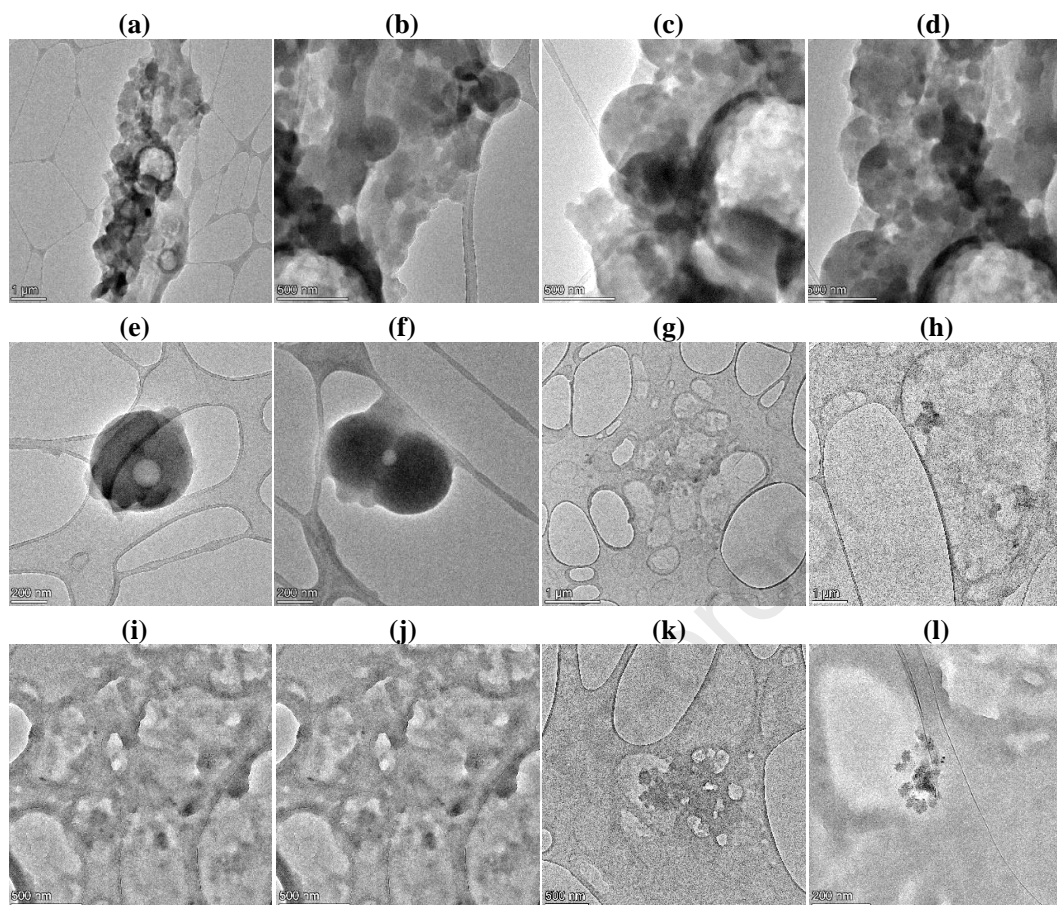


**Figure 3.** Detected elements by ICP-MS analysis of the digested solid matter.

Portions of brown viscous collected material were dissolved/dispersed in water (sample called RSW, meaning recovered solid dispersed in water) and in isopropanol (sample called RSI, meaning recovered solid dispersed in isopropanol) to be characterized by HR-TEM and TEM-EDX.

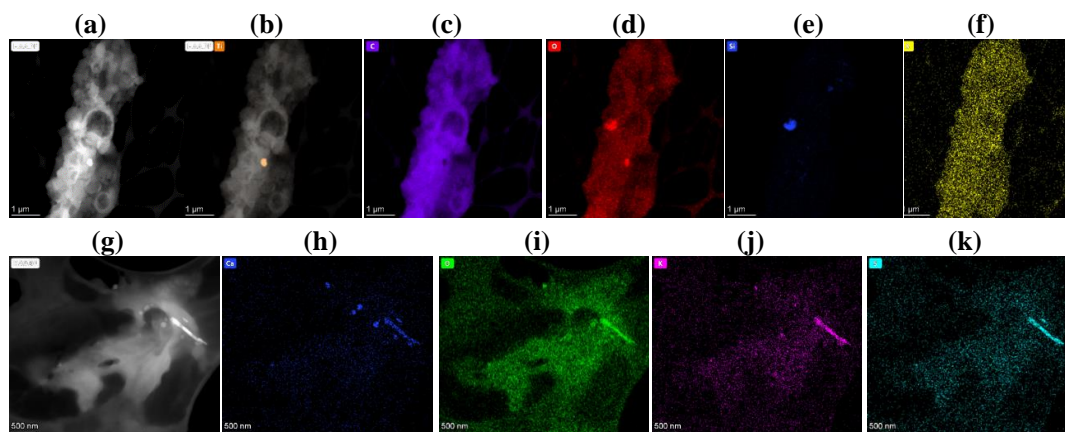
TEM images of the RSW sample (Figure 4 a-f) show agglomerated homogenous spherical structures. In contrast, in the TEM images of the RSI sample (Figure 4 g-l) few and small shapeless particles can be identified, and they seem to be composed by an insoluble shapeless structure, coated by a film which is likely formed by the precipitation of soluble compounds when isopropanol is evaporated. These results indicate that the solid matter deposited on the rubber piece is mostly water-insoluble, but highly soluble in isopropanol.





**Figure 4.** TEM images of the RSW (a-f) and RSI (g-l) samples (using, respectively, water or isopropanol as dispersants/solvents).

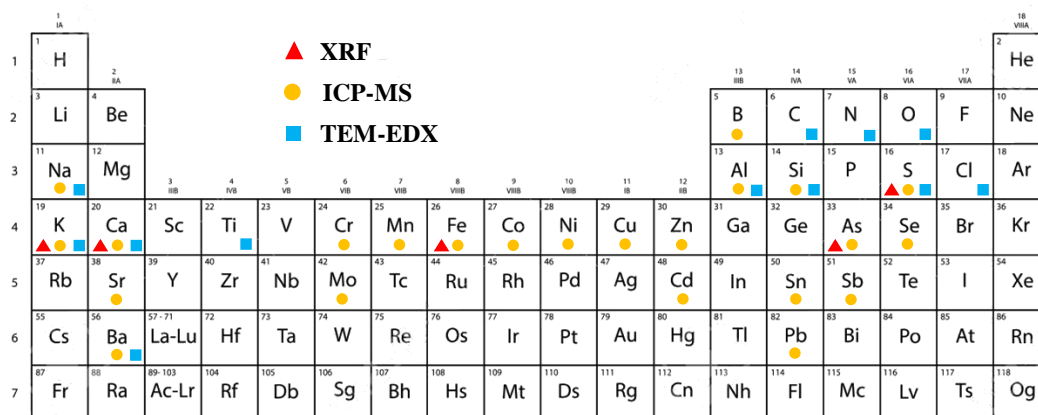
The TEM-EDX elemental mapping images and the corresponding HAADF TEM image of the structures observed in the RSW sample (Figure 5 (a-f)) reveal the presence of titanium (Ti), carbon (C), oxygen (O), silicon (Si), and sulfur (S). S, C, and O were detected in all the particles, homogeneously and well-dispersed, while Ti or Si was only present in some particles. The calculated mass fraction (Figure S2(a) in supplementary information) shows that the collected solid matter is mainly composed of carbon (C) and oxygen (O), whereas silicon (Si), sulphur (S), potassium (K) and titanium (Ti) are present in very low amount.



**Figure 5.** HAADF TEM image for the solid matter when water (a) or isopropanol (g) was used as dispersant/solvent. TEM-EDX elemental mapping images of detected elements of solid matter when water (Ti (b), C (c), O (d), Si (e), S (f)) or isopropanol (Ca (h), O (i), K (j), and S (k)) was used as dispersant/solvent.

TEM-EDX elemental mapping images of the RSI sample (Figure 5 (g-k)) show that it contains calcium (Ca), oxygen (O), potassium (K), and sulfur (S). These elements are more homogeneously dispersed in the RSI sample than in the RSW one, and a higher number of small nuclei are found in the latter. As expected, particles are less defined and smaller in the RSI sample than in the RSW sample. This supports that the elements detected by TEM-EDX are more finely and homogeneously distributed when isopropanol is used as solvent/dispersant (RSI sample). Moreover, these observations agree and support the previous conclusion, which highlights that the collected solid particulate matter is more soluble in isopropanol than in water. C and O are the main elements detected, whereas Si, S, K, and Ca are found in a lower amount (see supplementary information, Figure S2(b)).

Considering the obtained results, it can be concluded that the solid matter collected from cigarettes tests is mostly composed of non-water soluble organic compounds (essentially formed by C and O). In addition, traces of other elements have been detected by XRF, TEM-EDX, and ICP-MS (Figure 6). S, K, and Ca are identified by the three mentioned techniques because, probably, they could be more homogeneously distributed throughout the sample. However, some elements have only been detected in some of the used techniques because they are found in a heterogeneous way and/or not in sufficient quantity for the detection sensitivity of each technique (for example, Fe and As are detected by XRF and ICP-MS and Na, Al, Si, and Ba from ICP-MS and TEM-EDX).



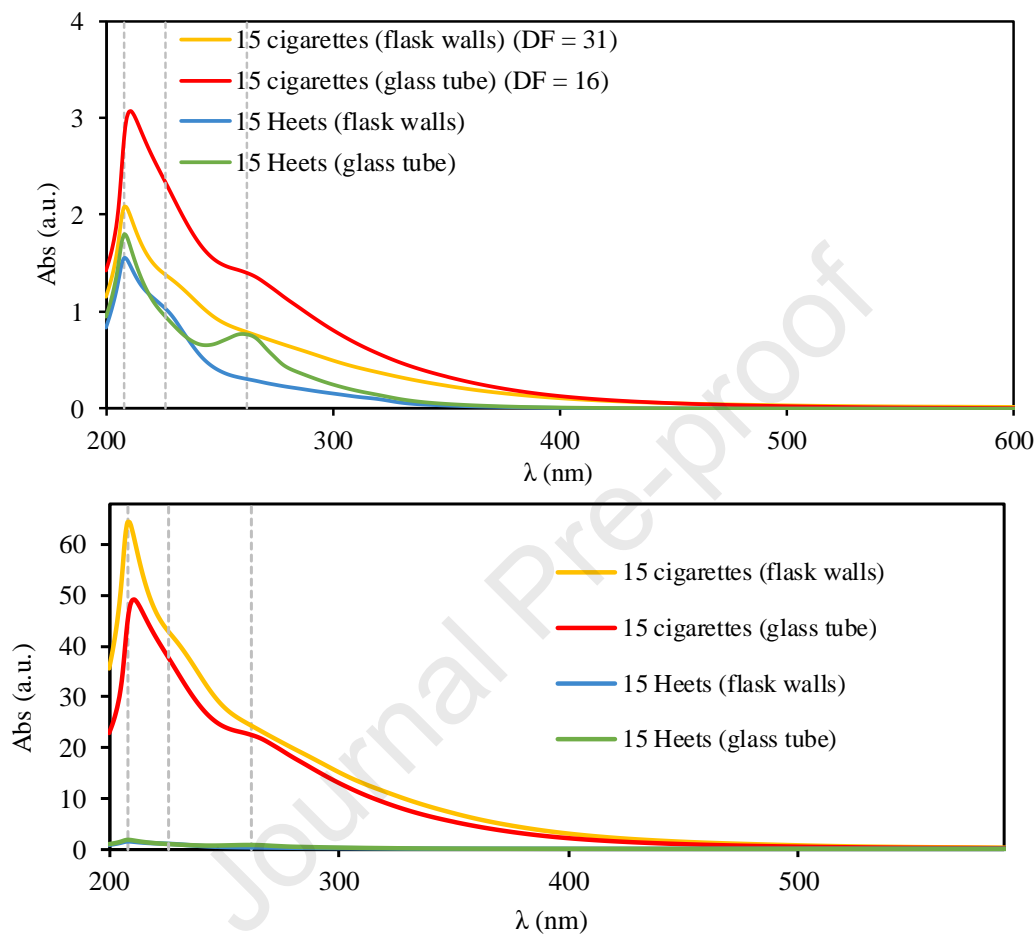
**Figure 6.** Periodic table of the elements where those detected by different techniques in the solid matter collected in the rubber piece are marked.

### 3.2. Particulate matter deposited on the glass tube and on the flask walls (parts 1 and 3 of the experimental setup)

UV-vis absorption spectra of the filtered isopropanol solutions obtained collecting the matter deposited on the flask walls and glass tube (experiments simulating puffs with 15 cigarettes or 15 Heets) were obtained. Isopropanol was used as a blank. So, in each plotted samples the spectrum of isopropanol had been previously subtracted. The solutions from cigarettes' tests were too concentrated, leading to saturated signals, and they had to be diluted. Thus, Figure 7a shows the UV-vis absorption spectra of the filtered isopropanol solutions and, in the cigarettes samples, the dilution factor (DF, volume of the resulting solution divided by the volume of the concentrated solution) is indicated in the legend. In all cases, an intense peak at 210 nm has been observed. The samples collected from the glass tube show a broad peak at 260 nm, while the samples collected from the flask walls present a peak at 226 nm, meaning that although having many similarities they seem to have a different composition. Probably compounds of higher molecular weight condense on the glass tube, while lighter substances remain on the stream (part of them become trapped in water and most of those, still present in the aerosol stream, are deposited on the flask walls).

It is evident that the filtered solutions from cigarettes' tests are much more concentrated than those from Heets's ones (pay attention to the dilution factors). To better appreciate this, Figure 7b shows a comparison of the absorbance in which the cigarettes' curves have been replotted considering the dilution performed. These results mean that cigarette

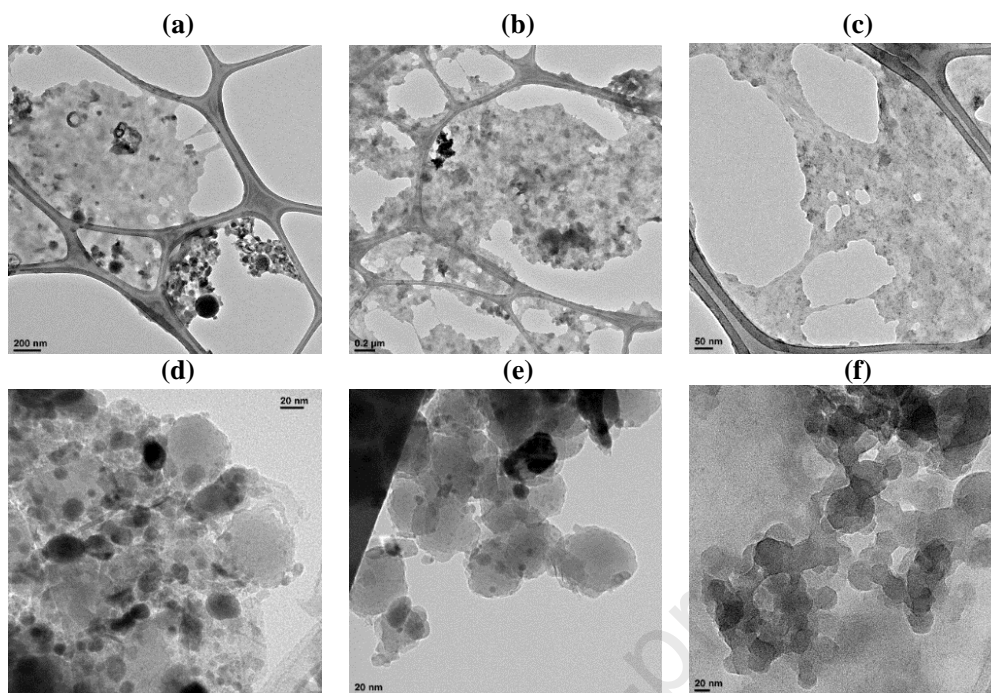
smoke contains a much larger content of isopropanol-soluble compounds, which could be deposited on hydrated tissues.



**Figure 7.** A) UV-vis absorption spectra of samples collected in isopropanol from flask walls and glass tube after tests with 15 cigarettes or 15 Heets. Samples from cigarettes' tests were diluted (dilution factors (DF) are indicated in the legend). B) Comparison between the previous series after multiplying the absorbance data for cigarette samples by their corresponding DF to properly compare results.

**Figure 8** compiles representative TEM images (with different magnifications, see scale bars in the images) of the samples collected on the flask walls (from 5 cigarettes) in which some insoluble spherical particles embedded in a soluble shapeless structure can be observed. Their size ranges from 5 to 100 nm, being more abundant particles with sizes up to 20 nm.





**Figure 8.** TEM images collected from flask walls of 5 cigarettes simulating puffs experiment.

In order to know the sizes of the particles collected in isopropanol from the glass tube and flask walls when cigarettes were smoked or Heets were used, laser diffraction was used. [Figure 9](#) shows the size distribution (density distribution,  $q \cdot 3$ ) and the cumulative distribution (Q3). The curves obtained are an average of the measurements of four different portions of each sample (see the size distributions with the standard deviation errors in Figure S3 in supplementary information). Note that the information provided in these plots is related to the relative contribution of each particle size range, but not with the absolute quantity of particles of that size range.

The results reveal that, as shown in [Figure 9](#), some particles are detected in both samples, cigarette and Heets-derived ones. The size distribution plots (upper insert in [Figure 9](#)) display differences in the sizes of particles contained in the smoke from cigarettes or aerosol from Heets deposited on the glass tube or on the flask walls of the experimental setup. In most samples, a bimodal distribution is observed. However, the mean particle diameter of each mode varies in each sample. From these data, it can be observed that the sizes of particles from samples coming from flask walls are more homogeneously distributed among the two modes than in the glass tube.

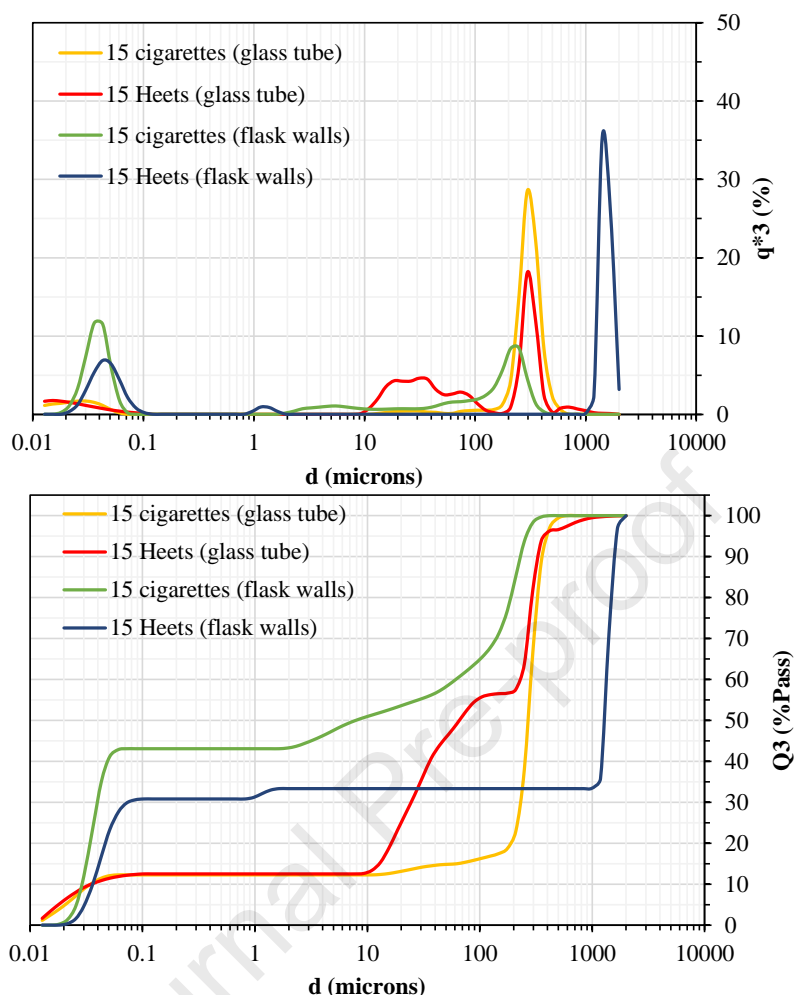
Figure S3 provides information about the particle mean sizes and deviation, showing that cigarettes-derived samples have much lower standard deviation values than the analogous Heets-derived samples. This higher size dispersion in Heets-derived samples implies that the processes leading to the appearance of these solids would be different in Heets in cigarettes, and may also be affected by the lower concentration of particles in Heets samples.

Mean particle size has been calculated for each sample considering the particles accumulation in each mode.

The highest mean particle size has been obtained for the Heet-derived sample coming from the flask walls (1020  $\mu\text{m}$ ), whereas the analogous cigarette-derived sample shows the lowest mean particle size (85  $\mu\text{m}$ ). This big difference in sizes would indicate that the particle sizes for cigarette smoke are typical of smoke particles generated during combustion, whereas the particle sizes in Heets aerosol are most likely coming as a result of the condensation of organic compounds or from dust or fragments of tobacco material carried into the aerosol by the air flow during puffing.

In a previous work [16], we have paid attention to particles from Heets and cigarettes collected in water showing that, in comparative experiments, the number of particles and quantity of insoluble compounds in water was much higher in cigarettes than in Heets experiments. In the case of cigarettes experiments, the generated particles were partially dissolved in the water flask. These results highlight the different nature of particulate matter collected from cigarettes and Heets and agree with the lowest mean particle size in cigarette-derived samples just remarked since some of these particles in Heets samples would only appear if high concentration would be reached in the flask, which is not the case.

With respect to the samples coming from the glass tube, the mean particle sizes were close (171  $\mu\text{m}$  and 265  $\mu\text{m}$  for Heets and cigarettes-derived samples, respectively), although as observed by UV-vis, TEM or ICP-MS these particles have a very different nature. To explain these results, it should be recalled that when smoke or aerosol passes through the setup, it first “interacts” with the (cold) glass tube, where some compounds can condensate. Then, the stream bubbles through water and, after this new interaction, deposition on flask walls takes place. This last deposited matter is not necessarily equal to that condensing in the glass tube, as it occurs.

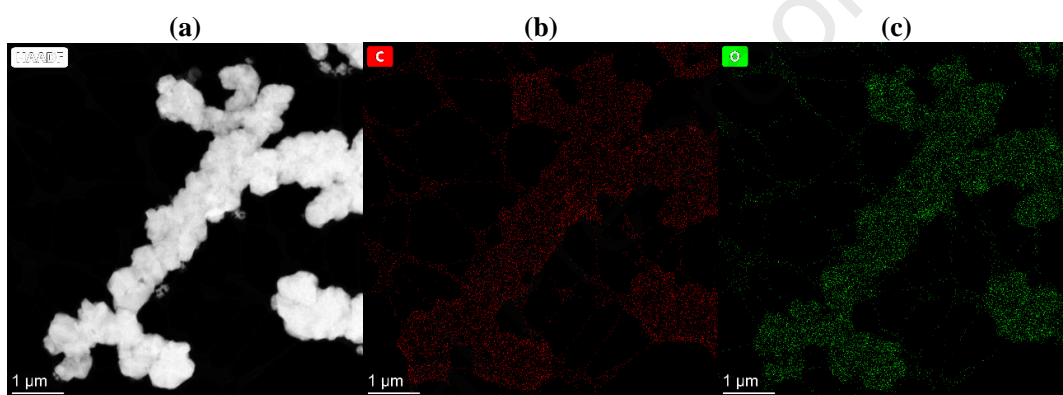


**Figure 9.** Size distribution of deposited solid particles on the glass tube and on the flask walls from cigarettes smoke and Heets aerosol. The upward figure represents the solid particle size distribution whereas the downward figure the cumulative size distribution.

Following these results, it can be concluded that, in both types of experiments, cigarettes and Heets ones, particles are emitted. However, in the Heets-experiments particles appear in much smaller quantities and also present different chemical compositions, with different volatility and chemical composition (i.e. metals). Both when simulating smoking of cigarettes or use of Heets, some particles are condensed and deposited in the first part of the set-up, the glass tube, giving rise in both experiments to particles of similar sizes. However, differences between deposits from cigarettes and Heets are noticeable in other parts of the set-up. These differences can be explained as a consequence of the different solubility in water of cigarettes and Heets-derived particles. Thus, the size of the particles found in the sample collected in the flask walls is much larger for Heets than for

cigarettes, because they are practically insoluble in water and remain in the aerosol stream, and become deposited on the flask walls.

The TEM-EDX elemental mapping images obtained for the sample collected on the glass tube after a 15 Heets test (Figure 10) show that they are mainly composed of C (73-68 wt. %) and O (31-26 wt. %), and both elements are homogeneously distributed, what would be in agreement with compounds of organic nature collected in this first part of the setup (quantitative data are shown in the supplementary information, Figure S4). These results also agree with other publications, which state that the composition of the mainstream aerosol from Heets is mainly glycerol, water, and nicotine [12,26].



**Figure 10.** HAADF TEM image (a) and TEM-EDX elemental mapping images (b, c) of samples collected from the glass tube after 15 Heets experiments.

The GC-MS analysis performed over the samples collected from the glass tube (Figure S5) reveals that in the cigarette samples, GC-MS detected the presence of 29 identified compounds. From them, 40% out of the 29 identified compounds corresponded to PAHs (this percentage was obtained by integrating the chromatogram curves). In contrast, 46 compounds were identified by GC-MS in Heets samples, and, from them, approximately 2% corresponded to PAHs. Therefore, despite a larger variety of compounds was found in the isopropanol solutions for Heets experiments, only a very small portion of them were PAHs, part of HPHCs (harmful and potentially harmful constituents). On contrary, for trapped cigarette smoke, fewer compounds were found, although a larger fraction of them was classified as HPHCs.



The presented results are in agreement with previously reported data, which also show that by using IQOS the PAHs release significantly decreases [11,27,28]. Thus, our study highlights that a lower number of PAHs are derived from Heets, in comparison with cigarettes but, in future work, a thorough characterization would be required not only to detect PAHs from the samples collected but also to quantify their corresponding concentrations.

Results of the ICP-MS analysis of samples collected from the glass tube after 15 cigarettes and 15 Heets are presented in Table 3. These data show that few elements have been detected (in comparison with the analogous analysis performed to solid collected in the rubber piece (Figure 3) and that they are the same in both samples, being Ca and Na the most abundant ones. The calculated ratio of the amount of each element in the cigarettes with respect to the Heets sample shows that Na and As are much more abundant in cigarettes samples, whereas Ca and Al are more abundant in the Heets ones.

**Table 3.** Concentrations of the detected elements by ICP-MS in samples collected from the glass tube after 15 cigarettes and 15 Heets tests and ratio of these concentrations.

	<b>15 cigarettes</b>	<b>15 Heets</b>	<b>Ratio</b>
	<b>simulating puffs</b>	<b>simulating puffs</b>	<b><math>C_{cig}/C_{Heets}</math></b>
	Concentration (ppb)		
<b>Na</b>	47.11	31.43	1.50
<b>Ca</b>	113.23	127.89	0.89
<b>Al</b>	0.42	1.240	0.34
<b>As</b>	0.07	0.05	1.33

With the purpose of quantifying the deposits collected on the glass tube, 30 mL of the collected isopropanol solution/suspensions were slowly evaporated at 50 °C (in an oven) by adding the liquid drop by drop to a crucible. This process took longer than 14 days. The residue originating from the cigarettes sample was yellowish and weighed 1.687 mg, while the analogous one originating from the Heets sample was non-colored and weighed 0.339 mg. Thus, the amount of solid matter deposited is 5 times higher for the cigarettes smoke than for the Heets' aerosol.

To deepen into the properties of the solid samples obtained after solvent evaporation, they were analyzed by TG-MS (10 °C/min, from room temperature to 950 °C in He flow 100

mL/min). It has been found that for both samples the largest mass loss occurs below 400 °C (see Figure S6, supplementary information), meaning that most compounds present in the solid residue are volatile or semi-volatile. Savareear et al. [29] have reported that the mainstream smoke from cigarettes and aerosol from Heets is composed, mainly, of acyclic hydrocarbons, aldehydes, monocyclic aromatic hydrocarbons, and heterocyclic compounds. These substances can be degraded to H<sub>2</sub>O, CO, and CO<sub>2</sub> by thermal treatment in an inert atmosphere. Evolved H<sub>2</sub>O, CO, and CO<sub>2</sub> have been quantified by mass spectroscopy (see Figure S7, supplementary information). The amount of these compounds evolved in cigarettes samples is 10 times higher in comparison with Heets samples. These results, together with the different compositions of particles derived from cigarettes and Heets previously shown, highlight the different nature and composition of the solid particles isolated from cigarettes and Heets. Thus, the amount of solid in Heets is much lower and its nature is different respect to the combustion related particles found in cigarette smoke.

### 3.3. Perspective for future studies

The topic and subject of this study is very broad and allows to be extended in different ways. Some of them are being commented next. As the study has focused on one brand of tobacco cigarettes and Heets (HTPs), it would be interesting to analyse products from other brands in order to complement these results. Precise identification and quantification (by using calibration samples) of the detected PAHs could be also a relevant target. Finally, risks assessment could also be a wide and interesting research task that would involve interaction and cooperation with experts in the field of biological and medical analysis.

## 4. Conclusions

Experiments conducted with cigarettes in continuous air flow have allowed collecting a viscous collected material on the rubber piece. About 80 wt. % of such substance is volatile or semi-volatile, being scarcely soluble in water and highly soluble in isopropanol, and it is mainly composed by C and O, although S, K, Ca, Fe, As, Na, Al, Si, and Ba have also been detected.

Puffing experiments with cigarettes leave some solid matter deposited on several parts of the puffing setup, which has been collected as a solution/suspension with isopropanol. These samples from cigarettes contain some insoluble particles mainly composed of C and O, but also by traces of S, K, Ca, Fe, As, Na, Al, Si, and Ba. In contrast, the analogous samples from Heets are formed by organic compounds (mainly composed of C and O) soluble in isopropanol. In addition, in Heets aerosol the number of PAHs from the total amount of detected compounds is much lower than in the cigarette smoke. A significantly lower amount of particles is contained in Heets-derived samples. The particle size distributions from, both, samples coming from cigarettes and Heets tests present a bimodal distribution. The particles deposited on the glass tube show a more similar and homogenous size distribution in both cases, since they come from condensation processes. However, in other parts of the setup, where the presence of particles and their size is affected by their solubility in water (flask), differences are observed: the mean size of particles collected from the flask walls is larger for Heets since they are mostly water-insoluble. This indicates that the particles found in Heets samples are of a different nature than smoke particles generated during combustion.

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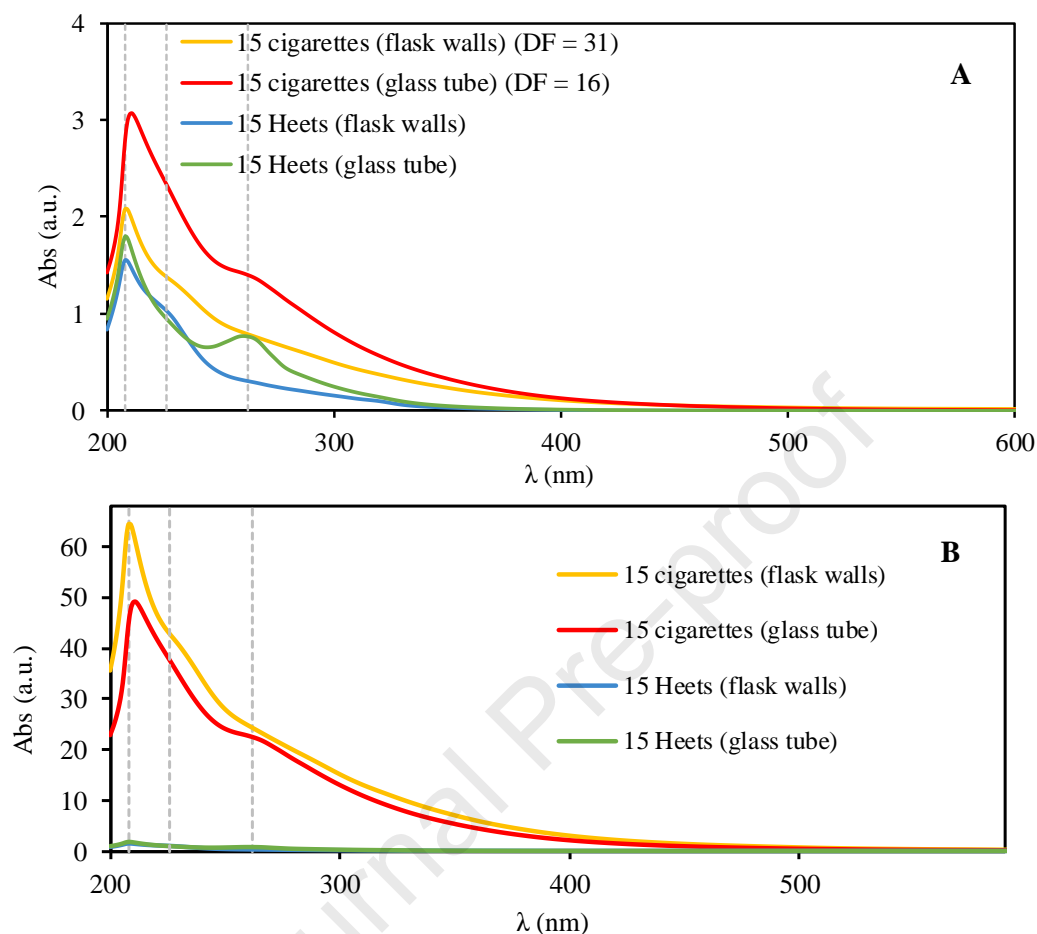
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**Figure 7.** **A)** UV-vis absorption spectra of samples collected in isopropanol from flask walls and glass tube after tests with 15 cigarettes or 15 Heets. Samples from cigarettes' tests were diluted (dilution factors (DF) are indicated in the legend). **B)** Comparison between the previous series after multiplying the absorbance data for cigarette samples by their corresponding DF to properly compare results.



### Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: