

Innovative methodology based on thermo-denuder principle for the detection of combustion related solid particles or high boiling point droplets: Applications to cigarette and the Tobacco Heating System THS 2.2

Pascal Pratte, Stéphane Cosandey, Catherine Goujon Ginglinger



PII: S0021-8502(17)30310-5
DOI: <https://doi.org/10.1016/j.jaerosci.2017.12.011>
Reference: AS5237

To appear in: *Journal of Aerosol Science*

Received date: 8 August 2017
Revised date: 20 December 2017
Accepted date: 25 December 2017

Cite this article as: Pascal Pratte, Stéphane Cosandey and Catherine Goujon Ginglinger, Innovative methodology based on thermo-denuder principle for the detection of combustion related solid particles or high boiling point droplets: Applications to cigarette and the Tobacco Heating System THS 2.2, *Journal of Aerosol Science*, <https://doi.org/10.1016/j.jaerosci.2017.12.011>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Innovative methodology based on thermo-denuder principle for the detection of
combustion related solid particles or high boiling point droplets: applications to cigarette
and the Tobacco Heating System THS 2.2**

Pascal Pratte*, Stéphane Cosandey, and Catherine Goujon Ginglinger

Philip Morris International R&D, Philip Morris Products S.A., Neuchâtel, Switzerland (part of Philip Morris International group of companies)

*Corresponding Author: Pascal Pratte, Philip Morris International, Research and Development,
Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland Tel: +41 (58) 242 2675 Fax: +41 (58) 242 2811
E-mail: pascal.pratte@pmi.com

Abstract

Combustion-related solid particles are known to potentially cause adverse effects on human health. In the scope of developing further the understanding on how combustion-related particles trigger biological responses in human, collecting them efficiently is key prior their characterization. To collect and characterize them from an aerosol, a methodology using a Dekati thermo-denuder operated at 300°C was established. The current work addresses the performance of the method by assessing its removal efficiency based on determined aerosol penetration for model solid particles and liquid droplets. The solid particle penetration was measured to be $79.4 \pm 7.3\%$ linked to the aerosol wall losses. To evaluate the ability of the thermo-denuder to remove liquid droplets, aqueous solutions of propylene glycol and glycerine were nebulized. From the measurements, the largest penetration value was measured to be $2.7 \pm 1.0\%$. As a result, the methodology limit of detection [1] was found to be 3.7% and the lower limit of quantification (LLOQ) 11.1%. Moreover, further experiments were conducted to ensure that liquid-coated solid particles could be distinguished from non-evaporated droplets to avoid data misinterpretation. To this end, ~70 nm-NaCl particles were coated with glycerin reaching a size diameter on the order of the micron. The experiments showed that the layer of glycerin-coated NaCl was removed entirely when passing through the thermo-denuder for the submicron size range. As an application of the methodology, the 3R4F reference cigarette smoke and Tobacco Heating System 2.2 (THS 2.2) mainstream aerosol were tested in the thermo-denuder. The data demonstrated that for 3R4F mainstream smoke, solid particles or high boiling point droplets were quantified far above the LLOQ (7.5×10^9 particles for LLOQ, $\sim 10^{12}$ particles quantified for 3R4F mainstream). On the opposite, for THS 2.2 mainstream aerosol, the penetration was overlapping

with the LLOQ within the experimental uncertainty. According to the current work and former published data, during the use of THS2.2, no combustion-related particles were observed. To conclude, the thermo-denuder technology is appropriate to detect/quantify solid particles or/and high boiling point droplets when combustion processes take place or for condensable aerosols.

Key words: aerosol, combustion-related particles, penetration, thermo-denuder

Introduction

During recent decades, owing to the development of industrial activities and growth of goods and people transportation, the emission of combustion-related particles in the environment was of key concern because of their substantial impact on outdoor and indoor air quality [2, 3]. Based on physico-chemical considerations, it is generally recognized [4] that organic materials generate ultrafine organic particles when incomplete combustion takes place [5] whereas their production is unlikely to occur when organic materials are heated without any combustion [6]. In particular, anthropogenic outdoor sources of combustion-related particles are from e.g. diesel engine exhaust and biomass burning e.g. coal/wood [7]. On the other hand, indoor sources are from e.g. lit candles or incense emissions [8] and cooking activities [9, 10]. The indoor and outdoor emissions produce fine suspended particulate matter PM_{2.5} and ultrafine particle fractions PM_{1.0} [11]. Among others, the rise in combustion-related particles have the consequence to change the Earth radiative forcing resulting in climate change [12], reduce visibility owing to light absorptions/scattering [13] and affect human Health [14, 15]. Because of their small

size, ultra-fine particles are defined as nanoparticles [16] that can penetrate deep in lungs, reach the alveolar region and deposits at the mucus interface owing to thermal diffusion. When deposited, the high specific surface area of the ultra-fine particulate matter releases adsorbed materials from the surface into the blood circulation [17]. Additionally, the deposited combustion-related ultra-fine particles can change lung functions by slowing down macrophages activities, promoting cell oxidative stress [18, 19] and triggering cardiovascular [20-22] disease via their translocation in the blood stream [15].

Generally, the smoke generated from bio-mass burning contains a mixture of combustion-related particle, liquid droplets, volatile gases suspended in a carrier gas [4, 11]. The separation of the different phases is not trivial. One method generally used to remove volatile gases while keeping the combustion-related particles in the aerosol stream is to operate a gas stripper or a so called thermo-denuder. The general limitation of this approach is that potential low volatile droplets might not be evaporated completely. Currently, the removal efficiency of gas strippers is not well known for low volatile droplets and the potential influence of the aerosol composition on gas stripper efficiency was not yet studied to the best of our knowledge. This question needs to be answered to evaluate to what extent the separation of combustion-related particles from droplets in an aerosol stream is possible. Moreover, an important parameter to consider is the wall losses of the particulate matter in gas strippers under specific experimental conditions, to make sure the amount of combustion-related solid particles can be corrected for this effect and subsequently quantified. The aim of the methodology is to define the limit of detection and quantification which may enable the identification of combustion-related particles

in test aerosols by removing liquid droplets and using a particle counter. In the current work, we decided to perform this validation using a commercial Dekati thermo-denuder and model aerosols composed of low volatile species such as propylene glycol and glycerin to measure to what extent liquid droplets could be removed for different formulations. Based on the quantified validation parameters, two aerosol matrices were chosen to test the methodology. The first was a smoke generated from the 3R4F reference cigarette and the second produced from the Tobacco Heating System THS2.2¹ [23]. In a recent publication[24], it was demonstrated that combustion-related particles were not observed when using a Scanning Electron Microscope (SEM) in contrast to 3R4F reference cigarette. This is corroborated by the fact that THS2.2 heats tobacco and produces an aerosol containing liquid droplets whereas the cigarette burns to generate a smoke, containing combustion-related particles and droplets. To verify the method performance, the thermo-denuder treated 3R4F smoke and THS 2.2 aerosol were compared to the established limit of detection/quantification of the methodology.

Methodology

General description

A Dekati thermo-denuder was chosen as the instrument of choice to remove liquid droplets from mainstream aerosol and smoke because of its particular construction and the possibility to operate it at a temperature up to 300°C. In fact, this high temperature was needed to enable the evaporation of glycerin, used as a model aerosol during the method development. Three distinct experimental phases were conducted to improve the current methodology based on concentration

¹ THS 2.2 is commercialized under IQOS brand name

measurements of surrogate aerosols. From the measurements, the penetration, which is the probability that an aerosol passes through a porous media without being removed, was measured [11]. In a first step, a thermo-resistant aerosol composed of solid particles (NaCl and SiO₂) was used to estimate thermo-denuder wall losses. Subsequently, the thermo-denuder efficiency to remove model liquid droplets from the aerosol stream was quantified using various formulation mixtures composed of water, propylene glycol and glycerin. Finally, the thermo-denuder capacity of removing liquid coatings covering solid particle seeds was tested using NaCl crystals coated with glycerin. The different tests were repeated over different testing days. This was to estimate the variability and limits of detection/quantification for the methodology to detect any solid particles present in aerosols composed of water, propylene glycol and glycerin.

Aerosol penetration

Aqueous stock solutions containing solid particles and mixtures of glycerin/propylene glycol formulations were nebulized from a Trust Science Innovation (TSI) Constant Output Atomizer 3076 described below in this publication. The model aerosol was conveyed to the thermo-denuder in two separate experiments when the thermo-denuder was operated at 30 and 300°C. The aerosol concentration was measured by a TSI Condensation Particle Counter (CPC) 3775 and an Aerodynamic Particle Sizer (APS) 3321 described below. To calculate the penetration, the aerosol number concentration measured at 300°C was divided by the result obtained when operated at 30°C. In the current work, the wall diffusion was found negligible at 30°C (data not shown) justifying to approximate aerosol penetration at 30°C close to unity.

Liquid coating removal efficiency

For the determination of the thermo-denuder capacity to remove the liquid coating potentially covering solid particle seeds, a TSI Condensation Mono-disperse Aerosol Generator (CMAG) 3475 containing glycerin was operated in two different phases. The first phase was set to generate only NaCl particles, to represent thermo-resistant nano-scale particle seeds. The aerosol was passed through the thermo-denuder and a TSI Scanning Mobility Particle Sizer (SMPS) model 3080 was used to measure the size distribution. In a second phase the CMAG was set to generate nearly mono-disperse glycerin-coated NaCl particles. The size diameter was measured using the SMPS at the outlet of the thermo-denuder for which the temperature was maintained at 30°C. In the same experiment, the temperature of the thermo-denuder was raised to 300°C and the resulting aerosol was measured by the SMPS. In these experiments, the APS was used to verify whether any droplets larger than one micron would still be present after the aerosol passage in the thermo-denuder at 300°C.

Thermo-denuder principle, aerosol generation and measurements

The Dekati thermo-denuder was selected to enable the removal of liquid droplets from test aerosols. The current work addresses the experimental steps which led to the improvement of the methodology by optimizing the experimental setup and providing a precise evaluation of the associated limit of detection [1] and lower limit of quantification (LLOD). The evaluation of the limit of detection and quantification was performed using model liquid droplets and solid particles. To conduct the measurements, two detectors were used. A butanol TSI Condensation Particle Counter (CPC) 3775 was operated at a volumetric flow rate of 1.5 l min^{-1} to count

particles/droplets below 3000 nm and a TSI Aerodynamic Particle Sizer (APS) 3321 was used at a volumetric flow rate of 5 l min^{-1} to count particles/droplets above 500 nm. The instruments were positioned at the outlet of the thermo-denuder as illustrated in the schematic of the experimental setup shown in Figure 1. According to the manufacturer of the Dekati thermo-denuder, the instrument should be operated at a total volumetric flow rate of 10 l min^{-1} . The total volumetric flow rate of the combined CPC and APS reached 6.5 l min^{-1} and an additional air flow of 3.5 l min^{-1} was needed and achieved using a primary pump.

Prior to starting an experiment, the thermo-denuder temperature was set at 30°C while compressed air circulated at $70 \pm 3 \text{ l min}^{-1}$ in the instrument casing to make sure the thermo-denuder gas stripper efficiency stayed constant over the course of an experiment. A TSI Output Atomizer 3076 was used to produce the liquid droplets and model solid particles. To generate the aerosol from aqueous stock solutions, a pressure of 2 bars was applied at the inlet of the COA. As a result, the liquid from the stock solution was nebulized and transported further using a TSI conductive tube. For the CMAG operation used for the glycerin-coated NaCl experiments, a total volumetric flow rate of 8 l min^{-1} was supplied at the inlet of the aerosol generator.

The thermo-denuder validation was carried out over five distinct days for each selected model aerosol. The APS and the CPC sampled continuously during the aerosol generation and data recording was performed every 10 seconds for the APS and 1 second for the CPC. For each testing day, the tested model aerosols are listed in Table 1 for solid particles and in Table 2 for liquid droplets. When the aerosol nebulization started, the COA was stabilized for 5 minutes followed by 5 minutes

sampling. This generated 30 replicates with the APS and 300 replicates using the CPC in this time period. This procedure was applied when the thermo-denuder was stable in temperature, typically 45 minutes after the temperature setting was applied. From all replicates, averages and standard deviations were calculated.

Application to cigarette smoke and Tobacco Heating System (THS 2.2) aerosol

3R4F cigarette mainstream smoke and THS 2.2 aerosol were generated in separate experiments using a Programmable Dual Syringe Pump (PDSP) using the Heath Canada regimen (55ml per 2 seconds puff, inter-puff 30 seconds, 12 puffs for 3R4F and 10 puffs for THS 2.2). A TSI dilution unit used upstream from the thermo-denuder was positioned to avoid its saturation, allowing liquid droplets to be removed efficiently. The LOD and LLOQ obtained in the current work were used to determine if any combustion-related solid particles or high boiling point droplets were detected/quantified for 3R4F smoke and THS 2.2 aerosol.

Results

Solid particle penetration and wall losses

Selectivity is the ability of the methodology to remove liquid droplets while minimizing solid particle wall losses. To investigate this, the penetration was measured for selected reference aerosols. In practice, the aerosol average number concentration was measured at the outlet of the thermo-denuder set at 300°C, compared to the value when the temperature was set at 30°C. The penetration should be the highest possible for solid particles (minimizing wall losses) and the lowest possible for liquid aerosols (maximizing liquid removal). Selectivity was also investigated using solid particles coated with glycerin to ensure the methodology allowed to distinguish

between solid particles covered by liquid layers and pure droplets. To perform these tests, a CPC and an APS were used concurrently. A TSI Particle Size Selector was fitted at the inlet of the CPC to ensure counted particles/droplets were framed in a size range from 30 to 3000 nm. In this situation, the APS was operated to verify whether particles/droplets counted in the CPC were smaller or larger than 500 nm.

To evaluate wall losses in the thermo-denuder for a poly-disperse aerosol, thermo-resistant NaCl and SiO₂ particles were assumed to be representative for nano-scale solid particulate matter generally found in combustion processes such as cigarette smoke [26]. Figure 2 shows an experiment where the NaCl aerosol number concentration was monitored using a CPC positioned at the outlet of the thermo-denuder operated sequentially at 30 and 300°C.

From Figure 3, it is observed that the highest CPC data variability was associated with the lowest aerosol concentration obtained by nebulizing the less concentrated SiO₂ stock solution. This behavior is explained because the penetration is a statistical parameter for which the statistical error depends on the inverse square root of counted events [11]. Additionally, according to the error bars from CPC data, no significant difference in penetration values was observed for the different tested SiO₂ particles in comparison to NaCl. When the APS penetration data are considered in Figure 3, low values are observed for low aerosol number concentration and small sizes. This is because tested sizes were falling out of the APS working range. This

statement is supported by the fact that the CPC and APS penetration values agreed when 500 nm SiO₂ particles were chosen for the largest aerosol concentration.

In summary, the penetration for nano-scale poly-disperse particles was found to be $79.4\% \pm 7.3$ (see discussion section for more details), meaning that wall losses corresponded to an average value of $\sim 20\%$. Stated differently, the average penetration for any test aerosol cannot be larger than 86.7% under our experimental conditions owing to wall losses. A test aerosol measured with a penetration of $\sim 80\%$ would mean that only solid particles/high boiling point droplets are present in the stream.

Liquid droplet penetration and thermo-denuder removal efficiency

From preliminary investigations [24], submicron glycerin droplets containing water were used to determine the thermo-denuder liquid removal efficiency. From these experiments, the liquid removal yield was found to be approximately 86%. However, for the application of the methodology to smoke generated from tobacco bio-mass combustion or aerosols from gas vapor phase condensation (e.g. THS 2.2 or e-cigarettes), the chosen model aerosol could contain water, propylene glycol and glycerin. From that perspective, formulations containing these compounds in different proportions were chosen to test the thermo-denuder removal efficiency (see Table 2 for the nebulized formulations). The resulting aerosol penetrations were measured using both the CPC and APS at the outlet of the thermo-denuder set at 30 and 300°C, in separate experiments. The resulting penetration values for the CPC are reported in measurements.

and were found smaller than 4% when considering the experimental error. The largest penetration value is attributed to the highest propylene glycol amount. The APS data were not considered in this case because measurements were overlapping with the background noise showing that no solid particles or droplets were quantified above 500 nm. The current data will be discussed further in the next section linked to the limit of detection and quantification.

Coating removal experiments

The thermo-denuder efficiency in removing liquid layers around solid particle seeds was tested for glycerin-coated NaCl particles according to Table 3. This was key to make sure the instrument was capable of removing not only liquid droplets but also liquid coatings covering potentially solid particle seeds. To test this, a CMAG was used to generate an aerosol containing NaCl seeds that may or may not be conveyed into a chamber saturated in glycerin vapors. When NaCl seeds by-passed saturated glycerin vapors, an SMPS measured a size distribution peaking around 75 nm after the aerosol was transported in the thermo-denuder at 30°C (see Figure , black lognormal-fitted curve). In a second experiment, NaCl seeds were mixed with glycerin vapor saturated at 140°C. In this case, glycerin vapors condensed in a controlled manner on NaCl seeds resulting in a quasi-mono-disperse distribution measured by the SMPS/APS (see Figure , dashed lognormal-fitted curve). The resulting size distributions peaked around 1000 nm because of glycerin layers covering NaCl seeds.

Finally, the thermo-denuder temperature was set at 300°C to test to which extent the glycerin coating could be removed from NaCl seeds. The result of this experiment can be seen in Figure , indicated by the dotted lognormal-fitted curve showing the size distribution peaking around 65 nm. From this, it can be concluded that glycerin coating was removed entirely from an aerosol containing solid particle seeds passing through the thermo-denuder at 300°C. However, during the first testing day (see Table 3), NaCl seeds covered by glycerin layers resulted in droplet size of approximately 2 micrometers. In this specific case, it was not possible to remove efficiently the glycerin coating when passing through the thermo-denuder set at

300°C. This finding indicated that submicron droplets only should be used in the thermo-denuder. Otherwise, diffusion driven mechanisms start being rate limiting in the thermo-denuder under our experimental conditions [26, 27].

Discussion

Repeatability and between day variability

Earlier in this publication, particles penetration was claimed to be $79.4 \pm 7.3\%$ for NaCl, calculated based on the estimated intra-day and between-day variability. The evaluation of these parameters was essential to make sure the methodology provided repeatable measurements on a given day and over different days. The equations used to perform the statistical analyses are displayed in Table 4 [28]. The penetration values, the intra-day and the between-day standard deviation are reported in Table 5 for solid particles used to characterize the thermo-denuder wall losses. The Table shows that for all tested solid particles, daily penetration variability was smaller than 3.5% from which the intra-day variability was rated as good. Furthermore, on a between-day variability standpoint, the between-day average penetration was varying from 72.2 to 92.3% with the largest between-day standard deviation being 7.3% for NaCl. Provided that the NaCl average penetration was falling in the middle of the measured range, it was chosen as the best model for poly-disperse aerosol to evaluate the thermo-denuder wall losses under our experimental conditions.

The glycerin-based aerosol liquid droplets used to characterize the removal efficiency of the thermo-denuder were evaluated in a similar manner than for solid particles. The calculated parameters are summarized in Table 6. On one hand, the highest measured individual penetration was found to be 4.4 % while the intra-day variability

was smaller than 0.1%. This led to the conclusion that all measurements were precise. On the other hand, the between-day averages were varying from 1.1 to 2.7% with the associated between-day standard deviation from 0.5 to 1.0%. This represents a relative standard deviation in the range 35-45%. This relatively large between-day variability was expected because it corresponds to the limit of detection of the method. Based on these findings, the minimum detectable penetration for the methodology was determined to be 3.7%.

We have demonstrated that when pure liquid droplets composed of water, propylene glycol and glycerin passed through the thermo-denuder at 300°C, the methodology allowed the determination that more than 96% in number were removed. In the case an aerosol is heterogeneous, solid particles may act as a seed for liquid coating to be formed while the removal is likely to be different in comparison to pure liquid droplets. To perform this investigation, removal efficiency experiments for glycerin-coated NaCl were carried out. In a first step, an SMPS was used to measure the size distribution of glycerin-free NaCl particles and the count median diameter was calculated from measured size distributions as reported in Table 7. The between-day average sizes were measured to be from 57 to 65 nm with an intra-day and a between-day standard deviation < 5 nm. This is a remarkable result because the maximum relative standard deviation was calculated to be 5%, inferring that the aerosol generation was stable. Over the same respective day, the aerosol generation was switched to glycerin-coated NaCl particles and passed through the thermo-denuder at 30°C. During day 1 the CMAG produced droplets with a size diameter of 2602 ± 13 nm in contrast to day 2 and day 3 for which the sizes were 1290 ± 46 nm and 1272 ± 13 nm, respectively. This discrepancy led to an artificially large between-

day standard deviation as reported in Table 7. When the thermo-denuder temperature was set to 300°C, the size diameter for day 2 and day 3 were reduced to 59 ± 1 and 58 ± 1 , respectively, very similar to glycerin-free NaCl particles. In contrast, for the day 1 when the size diameter of glycerin-coated NaCl was approximately 2 micrometers, the thermo-denuder was unable to completely remove the liquid layer. This is because the gas vapor phase diffusion is rate limiting for particulate matter larger than one micron and led to an artificially large between-day standard deviation.

Limit of detection, lower limit of quantification, upper limit of quantification

The limit of detection of the methodology was determined using the liquid droplet experiments by assessing the thermo-denuder penetration. For a mixture containing water, propylene glycol and glycerin, the penetration was found to be smaller than 4%. This lead to the conclusion that the thermo-denuder is capable of removing ~96% of the droplets while the remaining ~4% are the result of the combination of none-removed droplets and/or impurities. In the current work, this ~4% corresponds to an amount of $\sim 3.9 \times 10^4$ particles cm^{-3} detected by the CPC. This value was used as the background number concentration below which no detection is possible that is named the Limit of Detection [1]. From this, the Lower Limit of Quantification (LLOQ) is calculated as $\text{LLOQ} = 3 \times \text{LOD}$, translated to $\sim 1.2 \times 10^5$ particles cm^{-3} . The LLOQ is the minimum value that can be reported according to the methodology.

Application to 3R4F mainstream smoke and THS 2.2 aerosol

To test the methodology, THS 2.2 aerosol and cigarette mainstream smoke were generated in separate experiments using a Programmable Dual Syringe Pump (under Health Canada regimen: 55ml/2s, inter-puff 30 s, 12 puffs) using the setup

represented in Figure 1. The PDSP was coupled to a TSI dilution unit to avoid thermo-denuder saturation as described previously [24]. Figure 6 displays typical CPC experimental results obtained for THS 2.2 aerosol and 3R4F mainstream smoke. Each peak on the graph corresponds to a puff. The first series of peaks are attributed to the number of particulate matter objects counted for 3R4F smoke (black curve) and THS 2.2 aerosol (black-dotted) when the thermo-denuder was set at 30°C. The second series of peaks correspond to similar experiments performed when the thermo-denuder was operated at 300°C. 3R4F clearly shows peaks well above the LLOQ meaning that solid particles (~ 60% in number) or high boiling point droplets were present in the smoke. In the case of THS 2.2, the peaks were overlapping with the LLOQ, reflecting the experimental uncertainty. In a former scanning electron microscopy study [24], it was shown that combustion particles were neither observed nor detected in THS 2.2 mainstream aerosol. A matrix effect may explain this phenomena as THS 2.2 aerosol mainstream contains high boiling point compounds which could not have been completely removed in the aerosol mainstream. In addition, the removal efficiency of the thermo-denuder may depend upon other parameters such as the aerosol constitution and/or gas vapor phase partitioning. Further investigation of thermo-denuder parameters might be of interest to further evaluate its behavior when exposed to different aerosol types.

Conclusions

The current work addressed the performance evaluation of a Dekati thermo-denuder operated at 300°C performance. The removal efficiency was assessed using model solid particles and liquid droplets. The LLOD of the methodology was determined to be 3.7% based on model aerosols composed of propylene glycol, glycerine and water. To complete the assessment, glycerin-coated NaCl were used to show that,

for submicron aerosols, the liquid was removed entirely when passing through the thermo-denuder.

As an application of the methodology, the 3R4F reference cigarette smoke and THS 2.2 mainstream aerosol were tested. The data demonstrated that for 3R4F mainstream smoke, solid particles or high boiling point droplets were quantified far above the LLOQ (approximately 100 times larger than the LLOQ). On the contrary, for THS 2.2 mainstream aerosol, the penetration was overlapping with the LLOQ within the experimental uncertainty. These results demonstrated that no combustion-related particles were released and transferred in the mainstream of THS2.2. To conclude, the thermo-denuder technology can be used to detect/quantify solid particles or/and high boiling point droplets when combustion processes take place or for condensable aerosols.

Declaration of Interests

All authors were Philip Morris Products S.A employees at the time of the studies that were funded by Philip Morris Products S.A.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Reference

1. J. P. Lodge and T. L. Chen, *Cascade impactors: sampling and data analysis*. American Industrial Hygiene Association, 1988.
2. J. M. Arif, et al., *Modulation of macrophage-mediated cytotoxicity by kerosene soot: possible role of reactive oxygen species*. Environmental Research, 1993. **61**: p. 232-238.
3. F. Kheradmand, et al., *Autoimmunity in chronic obstructive pulmonary disease: clinical and experimental evidence*. Expert Review of clinical immunology, 2012. **8**: p. 285-292.
4. K. M. Butler and G. W. Mulholland, *Generation and transport of smoke components*. Fire Technology, 2004(40): p. 49–176.
5. T. Eidhammer, D. C. Montague, and T. Deshler, *Determination of index of refraction and size of supermicrometer particles from light scattering measurements at two angles*. Journal of Geophysical Research, 2008. **113**: p. 1-19.
6. K. C. Oha, C. B. Leea, and E.J. Leeb, *Characteristics of soot particles formed by diesel pyrolysis*. Journal of Analytical and Applied Pyrolysis, 2011(92): p. 456–462.
7. J. Buha, et al., *Physical and chemical characterization of fly ashes from swiss waste incineration plants and determination of the ash fraction in the nanometer range*. Environmental Science & Technology, 2014. **48**: p. 4765-4773.
8. W. R. Ott and H. C. Siegmann, *Using multiple continuous fine particle monitors to characterize tobacco, incense, candle, cooking, wood burning, and vehicular sources in indoor, outdoor, and in-transit settings*. Atmospheric Environment, 2006. **40**: p. 821–843.
9. G. Buonanno, L. Stabile, and L. Morawska, *Particle emission factors during cooking activities*. Atmospheric Environment, 2009. **43**(20): p. 3235-3242.
10. J. Gao, et al., *Determination of size-dependent source emission rate of cooking-generated aerosol particles at the oil-heating stage in an experimental kitchen*. Aerosol and Air Quality Research, 2013. **13**: p. 488–496.
11. W.C. Hinds, *Aerosol Technology*.
12. J. Hansen, M. Sato, and R. Ruedy, *Radiative forcing and climate response*. Journal of geophysical research, 1997. **102**: p. 6831-6864.
13. P. J. Grobicki, G. T. Wolff, and R. J. Countess, *Visibility Reducing Species in the Denver Brown Cloud I*. Atmospheric Environment, 1981. **15**: p. 2473-2484.
14. S. J. Klaine, et al., *Paradims to assess the enviromental impact of manufactured nanomaterials*. Environmental Toxicological Chemistry, 2007. **31**: p. 3-14.
15. C. Muhlfeld, et al., *Interactions of nanoparticles with pulmonary structures and cellular responses*. Am J Physiol Lung Cell Mol Physiol, 2008. **294**: p. L817–L829.
16. Vocabulary - Part 2: Nano objects -Nanoparticle, n.a.n., ISO/TS 80004-2 rev Nonotechnologies, 2012.
17. S. Steiner, C. Petri-Fink, and B. Rothen-Rutishauser, *Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms*. Arch. Toxicology, 2016. **90**: p. 1541-1553.
18. D. M. Brown, et al., *Size-dependent proinflammatory effects of ultrafine polystyrene particles: role for surface area and oxidative stress in the enhanced activity of ultrafines*. Toxicology and Applied Pharmacology, 2001. **175**: p. 191–199.
19. K. Donaldson, X. Y. Li, and W. MacNee, *Ultrafine (nanometre) particle mediated lung injury*. Journal of Aerosol Science, 1998. **29**: p. 553-560.
20. Glanz, S.A., *Air pollution as a cause of heart disease: time for action*. J Am Coll Cardiol, 2002. **39**: p. 943-945.
21. S. G. Klein, et al., *Endothelial responses of the alveolar barrier in vitro in a dose-controlled exposure to diesel exhaust particulate matter*. Particle and Fibre Toxicology, 2017. **14**: p. 1-17.
22. M. Rocha, et al., *Oxidative stress and endothelial dysfunction in cardiovascular disease. mitochondria-targeted therapeutics*. Curr Med Chem, 2010. **17**: p. 3827-3841.
23. M. R. Smith, et al., *Evaluation of the tobacco heating system 2.2. Part 1: description of the system and the scientific assessment program*. Regul. Toxicol. Pharmacol., 2016.

24. P. Pratte, S. Cosandey, and C. Ginglinger Goujon, *Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette* Human Experimental Toxicology, 2017. DOI: **10.1177/0960327116681653**: p. 1–6.
25. M. W. Fariss, et al., *Emerging Mechanistic Targets in Lung Injury Induced by Combustion-Generated Particles*. Toxicological Sciences, 2013. **132**(2): p. 253-267.
26. N. A. Fuchs and A. G. Suturin, *Highly dispersed aerosols*. Ann Arbor Science, 1970.
27. A. Fried, et al., *The reaction probability of N₂O₅ with sulfuric acid aerosols at stratospheric temperatures*. Journal of Geophysical Research-Atmospheres, 1994. **99**: p. 3517-3532.
28. Snedecor, G.W., *Statistical methods* eighth edition.

Tables

Table 1: Design of experiment for model solid particles nebulized using a TSI Constant Output Atomizer

| Stock aqueous solution concentration and particle types* | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|--|-------|-------|-------|-------|-------|
| NaCl | | | | | |
| SiO ₂ 100 nm (20µl/L) | | | | | |
| SiO ₂ 100 nm (40µl/L) | | | | | |
| SiO ₂ 100 nm (200µl/L) | | | | | |
| SiO ₂ 500 nm (20µl/L) | | | | | |
| SiO ₂ 500 nm (40µl/L) | | | | | |
| SiO ₂ 500 nm (200µl/L) | | | | | |

*solutions were prepared by mixing the amount mentioned in the table per liter of milliQ water.

Table 2: Design of experiment for model liquid droplets atomized using a TSI Constant Output Atomizer

| Stock aqueous solution concentration and formulation types | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|--|-------|-------|-------|-------|-------|
| 5 % in mass of the mixture Gly/PG (80%/20%) in water | | | | | |
| 5 % in mass of the mixture Gly/PG (50%/50%) in water | | | | | |
| 5 % in mass of the mixture Gly/PG (20%/80%) in water | | | | | |

Table 3: Design of experiment for glycerin-coated NaCl particle

| Compound | Parameters to be measured and calculated | Day1 | Day2 | Day3 |
|---|--|------|------|------|
| Glycerin-free NaCl particle | | | | |
| Glycerin-coated NaCl passing in the thermo-denuder at 30°C | Count Median diameter (nm) | | | |
| Glycerin-coated NaCl passing in the thermo-denuder at 300°C | Standard deviation (nm) | | | |

5 replicates

Table 4: Statistical parameters used to calculate the intra- and between-day variance

| Parameter | Definition | Equation |
|-------------|--------------------------------|--|
| S_i | Standard deviation for day i | $\sqrt{\frac{1}{n_i - 1} \sum_{j=1}^{n_i} (X_{ij} - \bar{X}_i)^2}$ |
| \bar{X}_i | Arithmetic mean of day i | $\frac{1}{n_i} \sum_{j=1}^{n_i} X_{ij}$ |
| \bar{X} | Global arithmetic mean | $\frac{1}{\sum_{i=1}^p n_i} \sum_{i=1}^p n_i \bar{X}_i$ |

| | | |
|-----------|----------------------|---|
| S_r^2 | Residual variance | $\frac{\sum_{i=1}^p (n_i - 1) S_i^2}{\sum_{i=1}^p (n_i - 1)}$ |
| S_d^2 | Day to day variance | $\frac{1}{(p-1)} \sum_{i=1}^p n_i (\bar{X}_i - \bar{\bar{X}})^2$ |
| S_L^2 | Between day variance | $\frac{S_d^2 - S_r^2}{\bar{n}}$ |
| \bar{n} | Average number | $\frac{1}{(p-1)} \left[\sum_{i=1}^p n_i - \frac{\sum_{i=1}^p n_i^2}{\sum_{i=1}^p n_i} \right]$ |

Table 5: Between-day average data associated to inter- and between-day variability for solid particle experiments

| Aerosol particles | Parameters | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Between-day average penetration (%) | Between-day standard deviation for penetration (%) |
|---------------------------------|-----------------|-------|-------|-------|-------|-------|-------------------------------------|--|
| NaCl | Penetration (%) | 69.7 | 76.9 | 87.7 | 77.1 | 85.8 | 79.4 | 7.3 |
| | Stdev (%) | 1.8 | 2.2 | 1.8 | 2.3 | 2.2 | | |
| SiO ₂ 100nm (20µl/L) | Penetration (%) | 85.1 | 100 | 93.6 | 98 | 84.8 | 92.3 | 7.1 |

| | | | | | | | | |
|-------------------------------------|--------------------|------|------|------|------|------|------|-----|
| | Stdev (%) | 1.2 | 1.5 | 2.1 | 3.3 | 1.5 | | |
| SiO ₂ 100nm (40μl/L) | Penetration (%) | 86.3 | 90.6 | 86.6 | 75.1 | 89.1 | | |
| | | | | | | | 85.5 | 6.1 |
| | Stdev (%) | 1.2 | 1.7 | 1.3 | 2.3 | 1.1 | | |
| SiO ₂ 100nm (200μl/L) | Penetration (%) | 76.2 | 80.5 | 79.2 | 81.2 | 81.3 | | |
| | | | | | | | 79.7 | 2.1 |
| | Stdev (%) | 2.5 | 1.0 | 0.9 | 1.8 | 1.7 | | |
| SiO ₂ 500nm (20μl/L) | Penetration (%) | 77.1 | 84.8 | 80.8 | 65.2 | 80.8 | | |
| | | | | | | | 77.7 | 7.5 |
| | Stdev (%) | 2.7 | 1.4 | 1.9 | 1.6 | 2.9 | | |
| SiO ₂ 500nm (40μl/L) | Penetration (%) | 67.5 | 75.5 | 81 | 64.2 | 76.4 | | |
| | | | | | | | 72.9 | 6.9 |
| | Stdev (%) | 1.9 | 1.7 | 1.0 | 2.6 | 1.8 | | |
| SiO ₂ 500nm (200μl/L) | Penetration (%) | 70.3 | 74.2 | 74.5 | 71.1 | 70.7 | | |
| | | | | | | | 72.2 | 2.0 |
| | Stdev (%) | 3.2 | 1.7 | 2.1 | 2.5 | 1.5 | | |

Table 6: Between-day average data associated to inter- and between-day variability for liquid droplet experiments

| Formulations | Parameters | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Between-day average penetration (%) | Between-day standard deviation for penetration (%) |
|--|--------------------|-------|-------|-------|-------|-------|---|--|
| 5 % in mass of the mixture Gly/PG (80%/20%) in water | Penetration (%) | 1.0 | 1.4 | 1.9 | 0.5 | 0.8 | 1.1 | 0.5 |
| | stdev | | | ≤ 0.1 | | | | |
| 5 % in mass of the mixture Gly/PG (50%/50%) in water | Penetration (%) | 1.3 | 1.6 | 2.6 | 0.7 | 0.9 | 1.4 | 0.7 |
| | stdev (%) | | | ≤ 0.1 | | | | |
| 5 % in mass of the mixture Gly/PG (20%/80%) in water | Penetration (%) | 2.3 | 3.0 | 4.4 | 1.7 | 2.2 | 2.7 | 1.0 |
| | stdev (%) | | | ≤ 0.1 | | | | |

Table 7: Between-day average data and to inter-/between-day variability for NaCl coating experiments

| Compound | Parameters | Day1 | Day2 | Day3 | Between day average size (nm) | Between day standard deviation for size (nm) |
|---|-------------------------------|------|------|------|----------------------------------|---|
| Dry NaCl crystals | Count Median diameter (nm) | 57 | 63 | 65 | 61.7 | 3.1 |
| | Stdev (nm) | | ≤ 1 | | | |
| NaCl coated distribution at the outlet of the thermo- denuder operated at 30°C | Count Median diameter (nm) | 2602 | 1290 | 1272 | 1721.3 | 762.6 |
| | Stdev (nm) | 13 | 46 | 13 | | |
| NaCl coated distribution at the outlet of the thermo- denuder operated at 300°C | Count Median diameter (nm) | 1436 | 59 | 58 | 517.7 | 6160.4 |
| | Stdev (nm) | 9 | 1 | 0 | | |

Figures

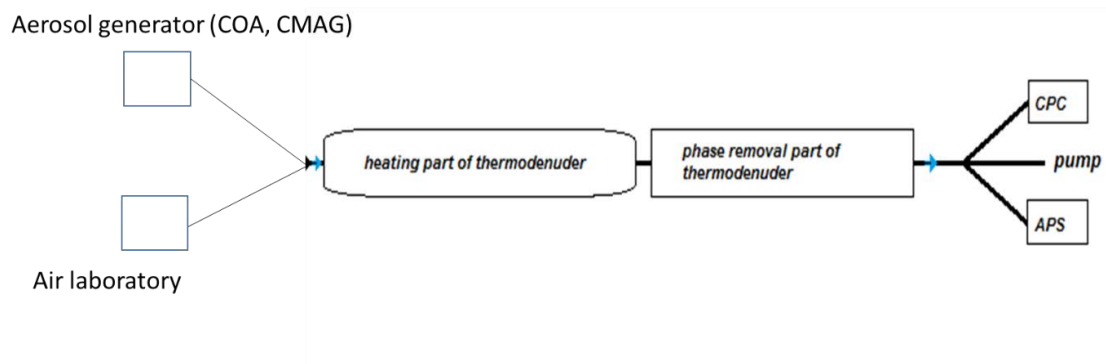


Figure 1: Experimental setup used to assess the thermo-denuder performance. The CMAG and the COA stand for Condensation Mono-disperse Aerosol Generator and Constant Output Atomizer, respectively.

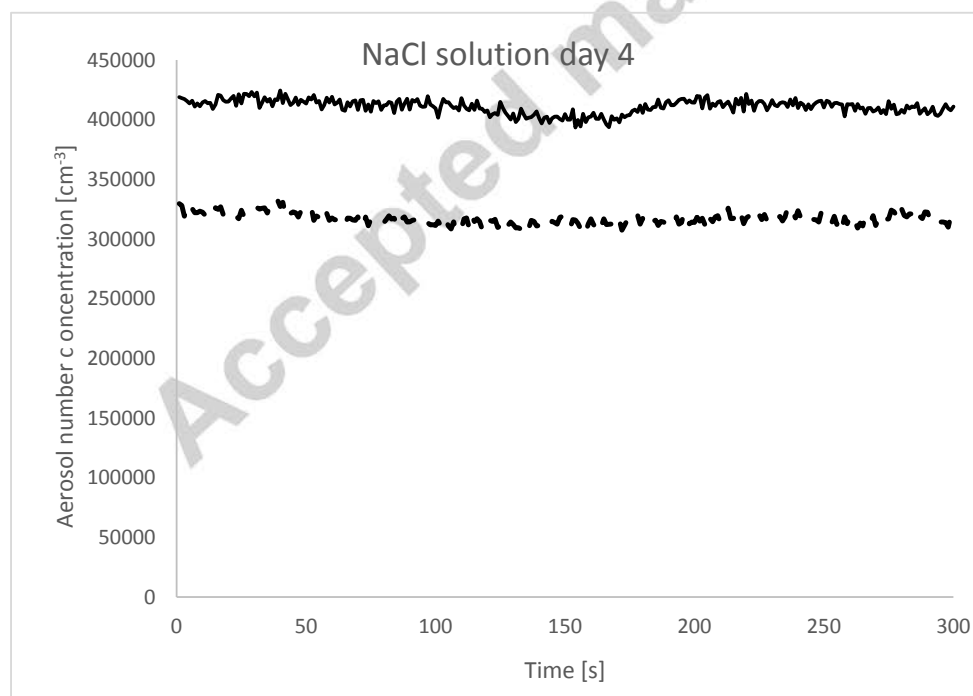


Figure 2: time-dependent aerosol concentrations measured with the CPC for NaCl particles. The continuous and dashed curves correspond to related data when the thermo-denuder was operated at 30 and 300°C, respectively.

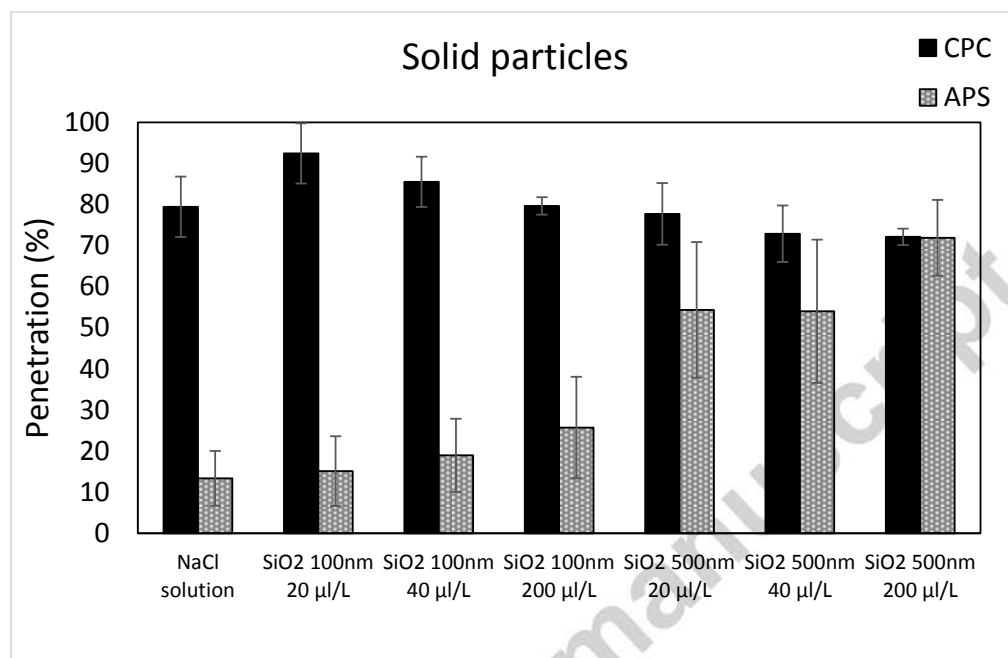


Figure 3: Solid particle penetration in percentage. The filled bars were obtained from CPC measurements. The dotted filled bars were obtained from APS measurements.

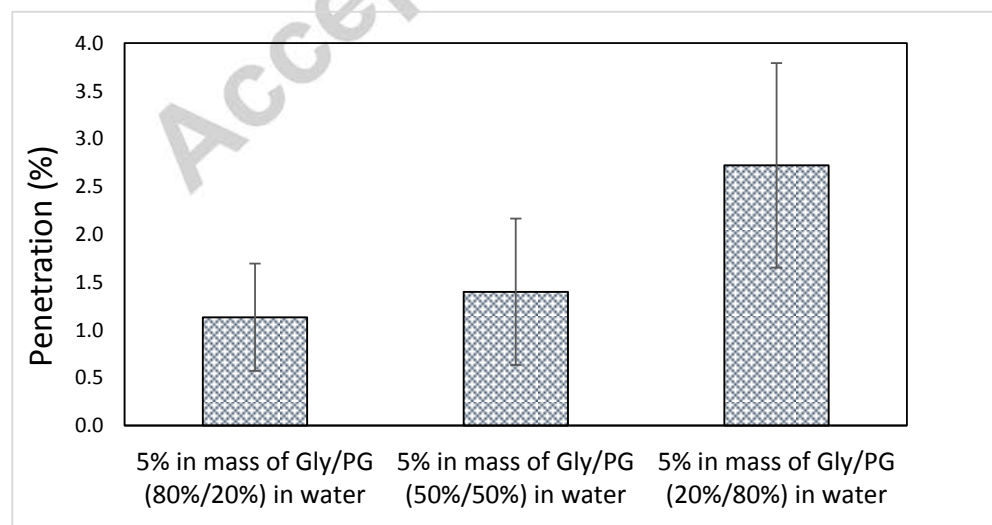


Figure 4: Penetration in percentage for tested nebulized liquid formulations when passing in the thermo-denuder operated at 300°C.

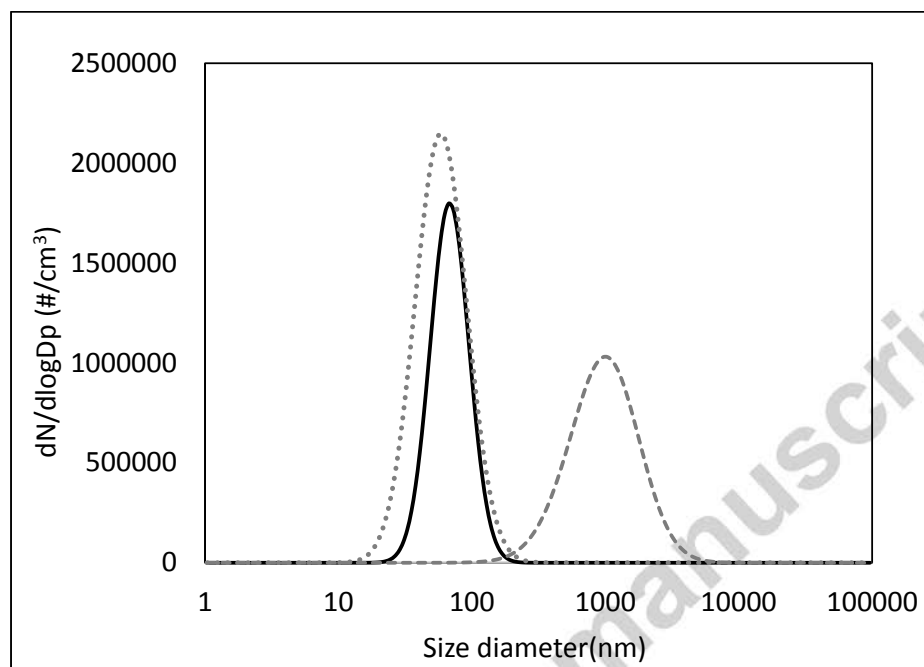


Figure 5: Coating removal experiments. The black lognormal-fitted curve corresponds to the measured size distribution of NaCl seeds when the thermo-denuder temperature was set at 30°C. The dashed and the dotted lognormal-fitted curves represent the size distribution of glycerin-coated NaCl seeds after they passage in the thermo-denuder when the temperature was set at 30 and 300°C, respectively.

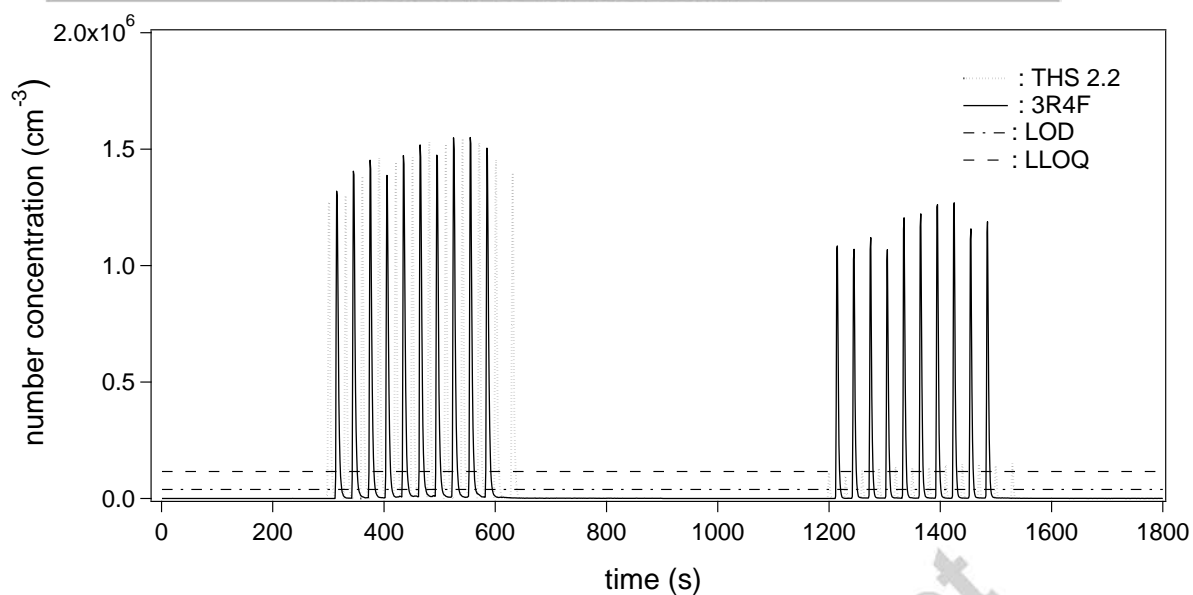


Figure 6: Typical example of measured puff profile for mainstream aerosol number concentration. The black curve corresponds to the aerosol number concentration for 3R4F mainstream smoke over 10 puffs, the grey-dotted curve to the mainstream aerosol for THS 2.2 over 12 puffs. The dashed-dotted and dashed horizontal lines represent the LOD and the LLOQ, respectively.

This manuscript addresses the following points:

- On a human health perspective, distinguish in an aerosol, combustion-related solid particles from liquid droplets.
- Propose a methodology using a Dekati thermo-denuder operated at 300°C to remove liquid droplets from an aerosol containing mainly glycerin, propylene glycol and water. From measurements determine the methodology limit of detection/limit of quantification
- Evaluate the methodology wall losses using thermo-resistant aerosols and CPC/APS as detection components
- Evaluate the methodology removal efficiency of model droplets containing glycerin, propylene glycol, water and CPC/APS as detection components
- Verify the ability of the thermo-denuder to remove completely liquid glycerin coatings from model solid particles
- Assess the methodology inter- and intraday variability to evaluate its robustness
- Test the methodology on 3R4F cigarette smoke and Tobacco Heating System (THS) aerosol
- Confront the methodology to SEM imaging (paper published in Human Experimental Toxicology in 2017, <http://journals.sagepub.com/doi/full/10.1177/0960327116681653>)