Real-time measurement of inhaled and exhaled cigarette smoke: Implications for dose

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Abstract. Inhalation of tobacco smoke aerosol is a two-step process involving puffing followed by inhalation. Measured smoke deposition efficiencies in the lung (20-70%) are greater than expected for smoke particles of 150 – 250 nm count median diameter (CMD). Various mechanisms have been put forward to explain this enhanced deposition pattern, including coagulation, hygroscopic growth, condensation and evaporation, changes in composition, or changes in inhalation behaviour. This paper represents one of a series of studies seeking to better quantify smoke chemistry, inhalation behaviour and cumulative particle growth. The studies have been conducted to better understand smoke dosimetry and links to disease as part of a wider programme defining risk and potential harm reduction. In this study, the average CMD of inhaled smoke was 160 nm while the average CMD of exhaled smoke was 239 nm with an average growth factor of 1.5.

1. Introduction
Tobacco smoke is a complex and dynamic matrix consisting of gaseous compounds and particulate material, in which over 4800 constituents have been identified [1]. The subsequent deposition of smoke constituents in the respiratory tract of smokers is an equally complex process both in terms of quantification of dose and location. Indeed an improved understanding of the dose and deposition location of tobacco smoke constituents in the human airways may well elude further information regarding the mechanisms of tobacco related diseases. The paper of Baker and Dixon [2] describes the work which has been carried out in the last century. The over-riding message from this paper is that there still remains a great deal of uncertainty as to the principal driving mechanisms governing tobacco smoke deposition in the human airways. A number of studies have also investigated the links between deposition and the physical properties of the aerosol, most notably the particle size of the inhaled aerosol. Richardson [3] noted that the retention of smoke particles in the human lung was higher than would be expected on the basis of their size when measured as they enter the mouth during smoking. Richardson suggested that the aerosol must grow within the humid environment of the human airways to alter the particle size distribution and therefore result in enhanced deposition [3].

The ambiguity which exists between experimental data and predicted data from deposition models [4, 5] has still not been fully addressed in the literature. Tobacco smoke particles are typically 150 – 250 nm CMD [6] and their high concentration (10¹² particles per cigarette) and the hygroscopic nature of the smoke droplets means that this particle diameter may change rapidly via coagulation and condensation [7,8]. Despite the small diameter of the smoke particles, smoke deposition efficiencies of 40-97% in the lung have been reported in the review of Baker and Dixon [2].
This paper describes a novel method to measure the puff by puff count median diameter (CMD) of inhaled smoke, post smoking by replicating the measured smoking profile, and also the puff by puff diameter of exhaled smoke in real time, therefore allowing a puff by puff growth factor to be calculated. This will define a boundary for the cumulative effect of growth and shrinkage of the smoke particles in the body. Particle deposition efficiencies were also measured, in separate exhale capture experiments using solanesol as a tobacco-specific chemical marker for smoke particles.

2. Methods

The inhaled and exhaled smoke diameters and solanesol deposition efficiencies were measured for seven volunteer smokers (5 males; 2 females) from whom informed consent had been obtained. These measurements were made with a research filter-tipped cigarette of conventional construction, using only Virginia tobacco, with an ISO tar yield of 8.8 mg per cigarette, measured as nicotine-free dry particulate matter (NFDPM). During smoking a pre-calibrated portable smoking analyser was used to record puff profiles (puff number, volume, interval and duration) for every experimental run.

Particle size and concentration measurements for smoke used a fast, electrical, differential mobility spectrometer (DMS-500, Cambustion, UK) at 10 Hz resolution [9, 10]. After each puff and inhalation, volunteers exhaled through a tube, from which a sub-sample of the total exhalate was taken into the DMS-500 particle spectrometer. The spectrometer sampling line requires a constant flow rate of 8.5 l.min\(^{-1}\) and therefore variable dilution of the exhaled smoke occurred. Thus, exhaled smoke particle size can be accurately measured at this time, but not exhaled particle number or mass concentration. Sample losses in transport to the spectrometer were calculated to be <0.1% for 150 - 270 nm CMD particles in an 8 mm diameter tube at 8.5 l.min\(^{-1}\).

Each volunteer smoked three replicates and inhaled to a depth of their choice, as per their normal smoking behaviour. This is referred to as ‘free smoking’, or VC\(_{\text{free}}\). Each volunteer took a puff and inhalation once a minute, and then immediately exhaled into the exhalate sampling line.

The inhaled particle size distribution was subsequently measured by re-smoking cigarettes on a smoking simulator (Smoking Cycle Simulator (SCS), Cambustion, UK) using human puffing profiles which had previously been recorded by the portable smoking analyser. The SCS has been described previously [10]. In short, the simulator continually delivers smoke and diluting air to the spectrometer via a critical orifice. At any time-point, flow through the cigarette, and primary dilution is regulated by altering the diluting air flow at 12.5 Hz resolution. Secondary dilution within the spectrometer is also controlled; thus mass is conserved in the sampling system and particle number and mass concentration can be quantified. The SCS was connected to the DMS500 spectrometer allowing the inhaled particle size distribution for each volunteer’s smoking profile to be measured. Measurements on each smoking profile were recorded in triplicate, giving nine inhaled profiles per volunteer.

For calibration purposes, the puff volumes generated by the SCS were compared against the portable smoking analyser quality control (QC) file, which consists of Bell, Triangle, Square and Near Triangle puffs at volumes of 25, 50, 75 and 100 ml. The QC file was played by the SCS with the smoking analyser used to record the actual puff volumes achieved. Figure 1 shows a snapshot of the agreement between the SCS volumes and the actual volumes from the QC file, while Figure 2 shows the good agreement between a complex human smoking profile recorded from a lighting puff, as recorded by the smoking analyser and as re-created on the SCS.

Particle deposition efficiency in the lung was estimated by Liquid Chromatography-Mass Spectrometry measurement of solanesol, a tobacco-specific high boiling point alcohol (C\(_{45}\)H\(_{74}\)O : MW = 630) which remains associated with the particulate phase of smoke [11] and represents approximately 3% of the particulate matter mass. For any cigarette type, the solanesol as a proportion of the particulate matter (by mass or UV measurement) remains constant at smoking flow rates from 1.05 to 4.5 l.min\(^{-1}\), covering measured human smoking flows. Inhaled particulate phase solanesol was calculated from solanesol measurement in the residual cigarette filter tip using filtration efficiency measurements from prior calibrations. Exhaled solanesol was measured from an exhale capture filter
pad. These two values were used to calculate particle deposition efficiency in the respiratory system for each test.

**Figure 1** SCS Profile shape and volumes

**Figure 2:** Human Double Puff Profile on the SCS

### 3. Results

Inhaled smoke particle size was calculated from a weighted average of the diameter of individual puffs and was found to be in the range of 150–175 nm CMD across the seven subjects. Equivalent exhaled smoke particle size in the range of 220–270 nm CMD with relatively consistent growth factors across volunteers, but with differences in absolute growth values between volunteers (1.3–1.8), consistent with measured smoking behaviour. The resultant mean particulate (solanesol) deposition estimate was found to be 49±17%. Table 1 shows the mean inhaled and exhaled CMD, the mean solanesol deposition and the mean growth factor from all volunteer measurements. There was also good consistency in terms of the inhaled CMD between the three replicates of each replayed smoking profile using the SCS. The average coefficient of variation for the inhaled CMD for nine measurements per subject (triplicate measures of each of three profiles) was found to be 3%.
Table 1: Summary data from all volunteer measurements

| Subject | Inhaled CMD (nm) | Exhaled CMD (nm) | Growth Factor | Solanesol Retention (%) |
|---------|------------------|------------------|---------------|-------------------------|
| 1       | 152.2 ± 4.1      | 267.3 ± 5.8      | 1.76          | 52                      |
| 3       | 157.5 ± 4.0      | 238.0 ± 3.1      | 1.51          | 62                      |
| 9       | 158.9 ± 5.9      | 227.4 ± 0.4      | 1.43          | 49                      |
| 12      | 161.8 ± 2.6      | 220.8 ± 2.2      | 1.37          | 21                      |
| 102     | 156.8 ± 5.1      | 260.3 ± 5.1      | 1.66          | 57                      |
| 105     | 174.4 ± 4.7      | 230.5 ± 3.9      | 1.32          | 67                      |
| 112     | 159.4 ± 3.9      | 227.9 ± 3.2      | 1.43          | 22                      |
| Mean    | 160.1 ± 6.4      | 238.9 ± 16.5     | 1.50 ± 0.15   | 47 ± 17                 |

An example of inhaled versus exhaled size on a cigarette-weighted average basis for each volunteer inhaling to a VC_free inhalation depth is shown in Figure 3. In general, normalized for all subjects, inhaled puffs became progressively smaller puff-by-puff as the residence time in the cigarette decreased, thus decreasing the time available for coagulation. This trend was significant and is shown in Figure 4. At the high concentrations in fresh inhaled smoke, coagulation is the principal mechanism for growth. In contrast, the particle size for all exhaled puffs normalised across all subjects was reasonably consistent from puffs 1-6, suggesting subsequent coagulation, hygroscopic, condensation and evaporation mechanisms in the lung were complete by the time of exhalation. In practice, this pattern suggested a progressive increase in growth factor puff-by-puff, observed in all subjects.

Figure 3: Cumulative frequency plots for inhaled (red) and exhaled (blue) particle size (CMD) for each volunteer

The data from Figure 3 represent the largest particles observed, but it is notable that > 90% of the exhaled particles are smaller than 500 nm CMD.
4. Discussion

The work carried out here was a study designed to test and validate new measurement tools to help understand particle size dynamics in the smoking process. This is important as observed particle deposition efficiencies (e.g. 47 ± 17% in this study) are significantly greater than those predicted by deposition models [4,5] of 10-20% for particles of the diameter of smoke. The models reflect deposition by a combination of impaction sedimentation and Brownian Motion, with a lung deposition minimum typically observed at approximately 500 nm. Various authors [12-14] have subsequently modelled particle growth through a combination of coagulation, hygroscopic growth, charge, breathing patterns, colligative (dense cloud) behaviour and hydrodynamic interactions with the surrounding air. These are addressed more fully in a recent review by Longest and Xi [15]. In their study, subsequent modelling of condensational growth suggested that smoke may grow to several microns in diameter, although this is not observed experimentally.

The cumulative growth factors of 1.3–1.7 measured here are consistent with previous published data [16] but do not achieve the cumulative growth calculated by modelling approaches. An alternative model suggests an alternative competing mechanism of particle shrinkage by evaporation and selective deposition of semi-volatile species, e.g. nicotine [17], and carbonyl species [18] which forms a significant proportion of the undiluted particle mass. If particle diameters become smaller, this would also enhance deposition relative to modelled data.

Our data reported here show clear evidence of particle growth of cigarette smoke from inhalation to exhalation. However, it is not yet possible to fully deconvolute these data to give a full understanding of the competing mechanisms involved at various parts of the smoking and inhalation cycle. Two areas in particular need further work. Further data is required to measure hold times in the mouth and lung and to measure inhaled and exhaled flow. It is also possible that selective deposition of the smaller particles may increase the exhaled d50 and enhance the measured growth factor.

In conclusion, the use of time-resolved electrical mobility analytical techniques is offering fresh insight into the aerosol behaviour of tobacco smoke. In turn, this further improves our understanding.

**Figure 4** - ANOVA general linear model fitted means for inhaled and exhaled particle size (CMD) against puff number
of the mechanisms driving regional deposition and local dose at each generation in the lung. A combination of these physical data, along with data on chemical composition and puffing and inhalation behaviour will allow a better understanding of effective dose and risk. Characterisation of these dosimetric processes offers scope for better understanding of sites and causes of disease, with appropriate dose application to in vitro models of disease.

**Ethical Considerations**

This study was conducted in line with the principles of the Declaration of Helsinki. Volunteers were all current smokers recruited from the workforce at BAT GR&D Centre. Each volunteer was interviewed and provided with study information prior to enrolment. Informed consent was obtained from each participant and each was made aware they were free to leave the study at any time.

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